Minnesota Dairy Health Conference

May 22-23, 2013

Minneapolis, Minnesota

University of Minnesota

College of Veterinary Medicine

UNIVERSITY OF MINNESOTA
EXTENSION

On behalf of the College of Veterinary Medicine, I would like to extend a warm welcome to you. The 2013 Minnesota Dairy Health Conference is part of the college's commitment to offering current research in practical contexts to both practitioners and producers. Your partnership in this educational process with us ensures improved management, healthy herds and a safer food supply.

Last year I was standing before you and could report to you about $700,000 grant from the U.S. Department of Agriculture's National Institute of Food and Agriculture that is sponsoring our National Center of Excellence in Dairy Production Medicine Education at the Dairy Education Center. The Center is located at the Davis Family Dairies' New Sweden Dairy, LLC, Nicollet County, Minnesota and shows a unique collaboration across several Universities: the University of Minnesota, the University of Georgia, University of Illinois, and Kansas State University. As of now the first two groups of students from 5 veterinary schools have successfully completed the intense 8 week curriculum.

Additionally, as you might know, our colleague Dr. Paul Rapnicki has left us in the previous summer. However, I am happy to inform you that we found a fabulous replacement: Dr. Gerard Cramer, who specializes in dairy cattle lameness and stockmanship. Welcome Gerard!

Today we are fortunate to offer a program featuring a group of leading dairy industry speakers from across North America. Joining this roster of presenters is a group I am especially proud to call my colleagues. Our dairy faculty and graduate students here at the University of Minnesota will further enrich the program by presenting their most current research.

In addition, I want to thank the sponsors and exhibitors of this annual conference. Your support makes this educational exchange possible. We especially appreciate the interest you take in our students' research, education and careers. I am fortunate to see the high level of quality in students entering the field of food animal veterinary medicine today and your mentorship and support of these students is critical to the industry. And finally, a special thank you to Dr. Riki Sorge for her committee's steadfast and visionary leadership of this conference. The conference's scientific program remains vibrant and timely due to the attention and involvement of our entire dairy faculty here at the University of Minnesota College of Veterinary Medicine.

Thank you for joining us.

Sincerely,

Trevor R. Ames, D.V.M., M.S., Diplomate ACVIM
Dean, College of Veterinary Medicine
University of Minnesota

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VETERINARY DIAGNOSTIC LAB

Laboratory for Udder Health

Committed to developing innovative approaches in laboratory diagnostics to help the dairy industry produce the highest-quality milk

The Laboratory for Udder Health (LUH) provides high-quality laboratory services to dairy veterinarians, dairy cattle producers, and other dairy industry professionals.

Located on the St. Paul campus of the University of Minnesota, the LUH is part of the Minnesota Veterinary Diagnostic Laboratory (VDL), an official laboratory for the Minnesota Board of Animal Health. The VDL is fully accredited by the American Association of Veterinary Laboratory Diagnosticians.

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The LUH is committed to protecting the dairy industry through the development of innovative approaches in laboratory diagnostics. Its goal is to provide reliable and fast services to improve udder health, reduce mastitis, and enhance milk quality and safety. The VDL's online result reporting system allows the LUH's clients to access test results in real time.

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UNIVERSITY OF MINNESOTA
College of Veterinary Medicine

WELCOME!

Delete this box or enter quote or tagline here

The College of Veterinary Medicine Alumni and Friends Society welcomes you to the 2013 Minnesota Dairy Health Conference, proudly hosted by the University of Minnesota College of Veterinary Medicine.

ABOUT THE CVM ALUMNI & FRIENDS SOCIETY

The Alumni & Friends Society (AFS) is the College of Veterinary Medicine's influential network dedicated to supporting, stimulating, and encouraging College of Veterinary Medicine students, alumni, and friends in their efforts to improve the health of animals and people through education, research, and industry advancements.

The AFS and its board of directors are involved in a variety of projects and activities that support education, research, development, and community service including scholarship, mentorship and awards programs. The College also brings alumni together at annual receptions at the AVMA and MVMA meetings and other veterinary conferences around the country.

JOIN US!

Whether you graduated from the University of Minnesota or just want to be an official friend, we hope that you will take this opportunity to support the Alumni & Friends Society by becoming an active member through the University of Minnesota Alumni Association. Membership is open to all graduates and friends of the College and members receive special benefits such as discounts on memberships, courses, and events, special rates on hotels and car rentals, and a subscription to Minnesota magazine. To join, visit www.MinnesotaAlumni.org/vetmed.
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Veterinarians care for animals at all stages of life.

SUPPORT THE NEXT GENERATION OF VETERINARIANS

The Alumni & Friends Society has established a direct scholarship fund for current students for positive, powerful and exciting learning opportunities. To make a contribution to the CVM Alumni & Friends Society Scholarship, please include fund #3177 in the memo field and mail your check to:

Alumni & Friends Society Scholarship
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WANT TO GET INVOLVED?

There are many ways to get involved - from joining the board to becoming a mentor to our students. We look forward to building our relationship with you by being part of your professional development and the communication bridge to your Alma Mater.
Jennifer Scholl - genz0005@umn.edu

Minnesota Dairy Health Conference

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Why Cows Die on Dairies

Franklyn Garry DVM, MS and Craig McConnel DVM, PhD
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Originally published in the AABP Proceedings:
Garry F, McConnel C. Why cows die on dairies. Proc Am Assoc Bovine Pract 45th annual meeting, Montreal, Quebec, Canada, 2012, pp82-86.

Abstract
On-farm death of adult dairy cows is a significant problem for both economic and animal welfare reasons. Adult cow mortality losses on dairies have increased in recent years. These losses and their causes are not carefully monitored or evaluated on most dairies leaving producers and veterinarians without the information needed to manage them. The reasons cows die are multiple and complex, necessitating an improved approach to diagnosis, information management and analysis.

Introduction
Death losses have not been studied very intensively in the dairy industry. Yet, mortality rates in the dairy industry are much higher than those in the cow calf or feedlot industries. Estimates of these death losses are variable. Unless they focus on monitoring cow deaths, dairy producers may underestimate the amount of adult cow death loss on their operations. The USDA:APHIS:VS National Animal Health Monitoring System (NAHMS) Dairy 2007 survey reported that 5.7% of dairy cows die on-farm across the country each year, an increase from 4.8% of the January 2002 inventory, and 3.8% of the January 1996 inventory.[14, 15]

Information from computerized dairy record systems suggests that mortality rates have continually increased over the last 10 years. In some states, adult cow mortality exceeds 10% per year.[2, 4] Few formal studies have focused on this issue, yet dairy cattle death losses are an extremely important problem. Not only are these losses an economic disaster, they also represent very substantial problems with animal well-being.

Adult cow death loss is an issue that should be very important to producers and veterinarians. But rising rates of occurrence across the industry suggests that veterinarians and producers do not have the information required to manage the problem appropriately. The purpose of this presentation is to critique the information we have, consider what information we need, and suggest changes in information gathering for dairy herds that would help diminish losses.

Why do dairy cows die?
Most studies of dairy cow mortality have come from outside the United States. Studies from the US on this issue have been primarily focused on culling and herd turnover rates rather than death losses per se. The 2007 national survey of dairies in the US[14] showed

that approximately 23.6% of dairy cows left herds permanently during 2007, and that approximately 5.5% of these cows were sold to other dairies, while 94% were culled (i.e. sold and not returned to milk production, sent for slaughter). The reasons cows were culled included reproductive failure (26.3% of culled cows), mastitis and udder problems (23%), lameness or injury (16%), other disease (3.7%), and poor milk production not related to these other problems (16%), while other miscellaneous reasons accounted for about 8% of culling. Therefore, on average, the overwhelming majority of dairy cows leaving farms are not fit for sale as dairy production animals, and approximately 50% of these cows leave because of disease or injury problems rather than being selectively removed because of low fertility or milk productivity.

Adult cow death losses appear to be attributable to reasons similar to those for culling cows. A recent literature review identified 19 studies between the years 1965 and 2006 that focused on dairy cow mortality in countries with relatively intensive dairy production.[13] While 10 of the 19 studies provided information about causes of death, none of the diagnoses were founded on necropsy evaluation. Only a single study discriminated between cows that were euthanized or died unassisted. The categories used to describe causes of death were relatively uniform across studies and were presented as: accidents, calving disorders, digestive disorders, locomotor disorders, metabolic disorders, udder/teat disorders, other known reasons, and unknown reasons. The NAHMS Dairy 2007 survey recorded causes of death similarly to those established through the literature review, documenting the percentage of cow deaths due to: euthanasia due to lameness or injury (20.0%), mastitis (16.5%), calving problems (15.2%), respiratory problems (11.3%), scours, diarrhea, or other digestive problems (10.4%), lack of coordination or severe depression (1.0%), poison (0.4%), other known reasons (10.2%), and unknown reasons (15.0%).[14]

Let's consider what the preceding information means. First it suggests that historically the careful tracking of causes of mortality on dairies has not been seen as a high priority. Such an attitude would make sense if deaths occur very infrequently and appear to have little to do with the health of the remaining herd. It makes a lot less sense when 5 to 10% of standing herd inventory is lost to death each year. This information also speaks to the diverse health challenges seen on dairies. Dairy cows are complex animals that go through multiple life stages in the course of their residence on a farm. This is very different than a beef feedlot where most of the animals are young and growing, somewhat equivalent to dairy heifers. In these populations infectious respiratory disease is far and away the number one health challenge that predisposes to euthanasia and death. For adult dairy cows there is no single predominant life-threatening disease.

It is also worth noticing that the categorization systems used on dairies and reported in the literature are not very helpful when it comes to instituting corrective actions. For example if you consider the category of lameness as a cause of death, there are so many potential causes of lameness that it would be difficult to institute a specific corrective action that would decrease the numbers in this category. Similarly, consider the wide range of disease problems that could be categorized as digestive death.

How good is our information about cause of death?
Cause of death entered in dairy record systems is usually based on producer assessment and diagnosis. It appears that most dairy veterinarians are minimally involved in the diagnosis of cause of death, and relatively few U.S. dairy operations perform necropsies in an effort to determine the cause of cow death. The NAHMS Dairy 2007 study reported that necropsies were performed on only 13% of operations and only 4.4% of cow deaths received a postmortem examination.[14] Therefore, historically almost all studies of dairy cow mortality are based on producer assessment rather than veterinary diagnosis and the causes of death are described using broad categories that do not provide much information about specific cause.

Dairy record systems appear to be an unreliable source of information concerning cause of death in individual animals. We have been studying the phenomenon of dairy cow mortality over the last several years. Our findings suggest that dairy producer assessment of the proximate cause of death is inaccurate approximately 50% of the time. Our results also validate that there are multiple causes of dairy cow death.[9] It seems reasonable to suggest that numerous health problems in dairy cows are not recognized early enough or treated appropriately to promote an optimal outcome, but this type of information cannot be retrieved from record systems. Furthermore, without good descriptors and records of the reasons that cows die, preventive measures that should decrease disease and death are not modified or improved to address the problem.

No specific reason has been identified for the increase in dairy cow death rates. In conversation with producers and veterinarians, some have questioned whether the federal regulations regarding down dairy cows and neurologic disease may have artificially increased recorded death rates. While this will contribute to recorded mortalities, death rates were increasing prior to the implementation of this rule.[11] Furthermore, if euthanized down cows represent more than a small fraction of dairy mortalities we need to ask why there are so many down cows that need to be euthanized. Others have suggested that specific disease problems such as hemorrhagic bowel disease may be increasing death rates. This could certainly be true on an individual dairy but the increased mortality rates across the industry exceed the incidence of any specific disease problem.

Any conjectures on the cause of increased mortality are difficult to validate without specific diagnoses. Determining the cause of death would provide invaluable information for preventing future deaths and improving herd health.[7] The fact that very few dairy cow deaths are evaluated by necropsy leaves a serious information gap in any analysis of cow mortality.

Epidemiological associations with dairy cow mortality
Although record systems as they are currently designed and used are not particularly helpful in managing adult cow death losses, they do demonstrate associations between high death rates and herd health problems. Analyses of large data sets demonstrate that herds with high rates of disease and culling also have higher death rates.[1, 3, 10] More specifically, high mortality in dairy herds is related to high rates of lameness and large

proportions of cows that are removed due to lameness or injury. Mortalities tend to occur much more frequently in the early part of lactation, coincident with increases in other health problems.[2] Death losses are related to the occurrence of respiratory disease, diarrhea, and mastitis.[10] These findings should not be surprising, as they suggest that herds that have poor ability to control lameness, injury, and infectious disease also have increased likelihood of cow death. It is important to recognize that these epidemiologic associations do not inform us of specific causes, and rather show that herds with certain types of problems also have higher rates of death. The problem for the producer and dairy consultant lies in how to determine specific actions that decrease disease prevalence and risk of death.

What can be done to decrease dairy cow deaths?

Most decisions in a low-cost production dairy model are made with input cost as the primary driving force, and potential negative impacts on the animals in the production system are seen as problems that must be managed as a consequence. For example, it is common that large scale expansion of a dairy will capture production cost efficiencies, but often with the caveat that expansions are accompanied by substantial problems with animal health. During the time that large numbers of animals are being imported to the herd it is routine that disease introduction is occurring. Numerous animal health problems are prevalent and even increase with time.[5, 16] Because there are compelling reasons for dairies to expand, there is a real need for the dairy industry and dairy veterinarians to reevaluate dairy management systems with a focus on optimum animal health.

An overview of the health challenges faced by dairy cows needs to recognize that some changes in the modern dairy industry may result in systematic problems with animal care. The labor force on most dairies is primarily composed of low wage workers without extensive, preexisting dairy cow management skills. The ability of dairy personnel to adequately identify disease in individual animals and respond with prompt individual animal attention is limited by the extent of their experience and training. The overwhelming majority of sick cows on dairies are identified, diagnosed, and treated by farm workers rather than veterinarians. Poor outcomes may be an issue of poor clinical disease management in addition to any preexisting problems with cow physiology.

Farm necropsy examinations should be an invaluable tool to help assess cause of adult cow death.[7] Necropsy of dead animals to assess and monitor cause of death is rarely performed on dairies.[14] This is in sharp contrast to other intensive livestock management systems, including poultry, swine, and feedlot enterprises where necropsy monitoring is routine. Most dairy veterinarians focus considerable effort on dairy reproduction, or udder health and milk quality, but little time on mortality evaluation. This presents a very significant liability to the dairy industry because efforts to effectively decrease mortality losses are hampered by a lack of monitoring and information necessary to accurately assess the problem.

We believe that dairy workers could be trained to more effectively monitor death losses, and to perform on-farm necropsy examinations in consultation with veterinarians when the veterinarian cannot be present to perform the examination on a freshly dead carcass.

We have presented this recommendation to producer groups and produced an on-line training program for that purpose on our website.[12] Very few producers or veterinarians have pursued this approach, attesting to the notion that monitoring actual cause of death has not been seen as a valuable pursuit.

Necropsy examinations provide good information, but we also need to develop new recording systems that allow the necropsy results to be recorded as usable information. On their own, necropsy diagnoses provide great detail about the specific cause of death, but do not necessarily provide information about why that specific cause occurred. Therefore necropsy information needs to be combined with other historical information about the affected animals to help direct management changes.[8] Our studies suggest that more than 50% of cow death losses are attributable to causes that could be mitigated with proper management.[8]

Because of the complex nature of dairy management systems a variety of causes are responsible for high disease and mortality rates, with different rates of occurrence on different operations. The wide range of lactational incidence risk for common diseases (milk fever: 0.03%-22.3%, RP: 1.3 – 39.2%, metritis: 2.2-37.3%, ketosis: 1.3-18.3%, LDA: 0.3-6.3, lameness: 1.8-30%) attests to the complexity of dairy systems.[6] To adequately address such complexity requires more accurate information about current losses, followed by management alterations that address the underlying problems. This will require changing the nature of information used in dairy management systems. An example of mastitis prevalence can illustrate this point. The specific infectious organism that causes a clinical mastitis episode can have a dramatic impact on outcome, and appropriate preventative or therapeutic measures need to be tailored to the specific cause, e.g. gram negative vs. gram positive, environmental vs. contagious, *Escherichia coli* vs. *Staphylococcus aureus*. Assessments and record systems that track "mastitis" without identifying other specific details provide less information than needed to establish effective interventions. Similarly, monitoring death losses with generic terms such as "lameness" or "mastitis" and performing this monitoring on the basis of presumption will not allow correction of management problems that may underlie the death.

Specific recommendations to decrease death losses

We have proposed an approach to monitoring death losses that should help producers identify management changes to improve cow health and survival.[8] The first step is to identify the magnitude of the problem on a dairy and commit to improving outcomes. Like any other substantial management change on a dairy, if the owner or manager is not committed to change it will not actually happen. Therefore simple analysis of the incidence of on-farm death and an assessment of its importance to the dairy and the well-being of the cows is critical.

Second, we recommend performing necropsy examinations to identify specific causes of death. This information needs to be considered along with other cow information such as preceding health problems, treatments, and individual cow circumstances as part of a complete post mortem evaluation. It is unrealistic to assume that 100% of all dead cows

will be examined by necropsy. Our experience suggests that routine necropsy examination is important but that targeting cases is useful. For animals euthanized due to obvious trauma, or where the cause of death is obvious based on priority veterinary assessment, necropsy examination usually will not provide much more information. Alternatively, for unexpected deaths or animals without simple specific antemortem diagnoses, necropsy can help not only define the cause of death but also inform farm workers about the types of problems that occur on the farm.

We have developed a conceptual model to help assign cause of death to categories that have more meaning than those simple categories that assign cause of death to an organ system that the owner perceives was affected by disease. Necropsy is a key tool for assigning cause of death, if the information obtained is also matched with other animal information. Dairy workers who are involved in animal care should be included in the discussion of the necropsy and cause of death. The monitoring and focus on cause of death as an important component of dairy animal monitoring increases owner and worker focus on the actions needed to prevent future death losses.

We recommend maintaining hard copy records of each case of death. When a particular category of death is seen to be problematic the details of the individuals in that category can be reviewed. As with all records, they need to be used to inform management if they are to be any use at all. Therefore we recommend periodic meetings between farm managers and veterinarians to consider death losses and what can be done to improve outcomes.

More focus needs to be placed on evaluating subclinical disease problems. One of the problems with current record systems is that health events are only entered when they are obvious and prompt a treatment. Subclinical disease does not fit this category and therefore information about subclinical cow problems cannot be retrieved to be compared with assessment of death losses. Consider for example the assessment of lameness on dairies. As noted above, high rates of lameness are strongly associated with high rates of death losses. However, most records systems monitor lameness only when cows receive specific treatment. It is unusual for dairies to do routine locomotion scoring that detects cows with more modest degrees of lameness. It is likely that management changes targeted to improving overall cow locomotion will also improve other aspects of cow health and ultimately lead to decrease death losses.

Conclusions

There will not be a single simple answer to the problem of high mortality on dairies. Steps toward managing this challenge will require recognizing and defining the problem, improving information systems to provide details necessary to take action, and monitoring appropriate metrics that promote ongoing attention to management corrections.

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AMERICAN FOODS GROUP

MINNESOTA DAIRY HEALTH CONFERENCE

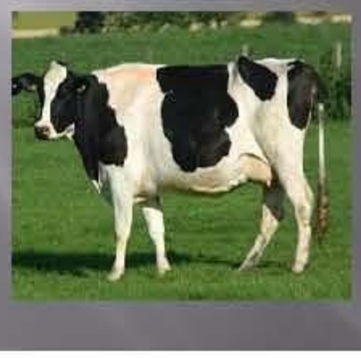
Minnesota Dairy Health Conference

May 22, 2013

AMERICAN FOODS GROUP

WHAT'S ON THE FORK?

More importantly how do help producers make sure it stays there?



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Dairy Cows are a Valuable Meat Resource

- Approx. 96% of dairy cows are harvested for human consumption
- Dairy Cows Account for 8% of U.S. Domestic beef production
  - 25% of the U.S. non-fed beef supply
  - 18% of the U.S. ground beef supply

Dairy Cows are a Valuable Meat Resource

- Key component to dairy producers income
  - Cull Cows
  - Calf sales for veal and fed beef
- Major supply for MN/WI meat processors
  - Approx. 70% of slaughter
- Creates 1000's of jobs
  - Marketing
  - Processing
  - Transportation

Challenges to Dairy Beef

- Driven by:
  - Consumers
    - Health and safety concerns
    - Moral and Philosophical Concerns
  - Special interest groups opposed to animal agriculture
    - HSUS
    - PETA
  - Consumer Health Groups

PETA

THE HUMANE SOCIETY OF THE UNITED STATES

Challenges to Dairy Beef

- Come in various forms:
  - Decreased consumer demand
  - Increased customer requirements
  - Increased regulation
    - Congress
  - Negative publicity
    - Media and blogs
  - Costly legal action
    - Personal injury lawyers

USDA FSIS

Challenges to Dairy Beef

- On March 14, 2009 President Obama announced the creation of a new Food Safety Working Group to advise him on how to upgrade the U.S. food safety system. The Working Group, chaired by the Secretary of Health and Human Services and the Secretary of Agriculture, is recommending a new public health-focused approach to food safety based on three core principles:
  - Prioritizing Prevention
  - Strengthening Surveillance and Enforcement
  - Improving Response and Recovery

Challenges to Dairy Beef

- This framework is a coordinated response to ensure food safety activities, and helps agencies like FSIS to target areas in the farm-to-table continuum where more attention is needed. One broad area within this continuum that FSIS is focusing on is strengthening policies around pre-harvest controls.

Challenges to Dairy Beef

- So what does this all mean to us?
  - We need address the issues as an industry to prevent some further regulation and to address the regulations moving forward.
- Do we let dairy cows go the same way as the poultry industry?
  - We have much more at stake!

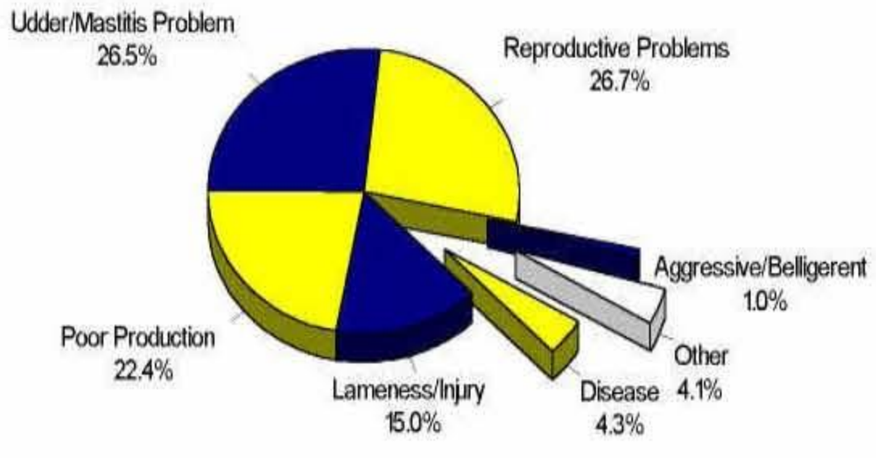
American Meat Institute

- The packer/processor should engage producer associations to develop pre-harvest programs that address food safety issues both microbial and chemical
- Some producer groups, pork specifically, have defined and implemented programs (PQA+). Most AMI pork packers require PQA+ as a part of purchasing programs
- A focus of National Advisory Committee on Meat and Poultry Inspection and FSIS.
- When needed, HACCP plans can reflect the use of these supplier programs
- Support timely approval of pre-harvest interventions

Issues for Dairy Producers

- Quality concerns
  - Direct economic impact
  - May have impact on other issues
- Chemical contamination
- Microbial contamination
- Humane animal handling

Percent of Dairy Cows Culled for Slaughter by Reason for Culling



Quality Concerns

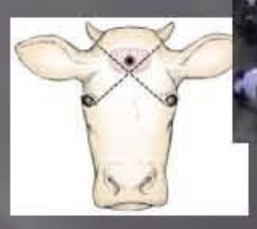
- Lameness
- Downers
- Body Condition Scores
- Transportation
- Bruising

Addressing Quality Concerns

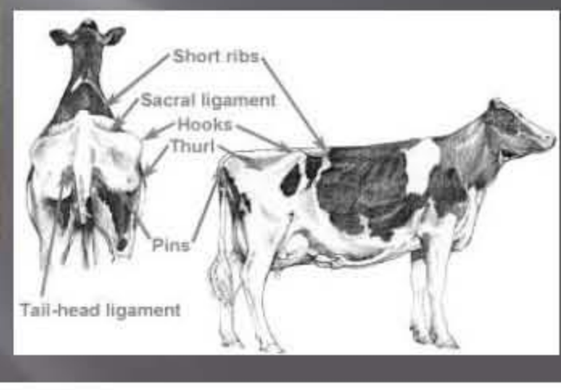
- Market animals timely
- Use broader analysis for making marketing decisions
- Choose the best market method depending on the cow's condition
- Evaluate and change transporters
- Evaluate and modify your facilities

- Lameness
  - Increases risk of downer animal
    - Must walk unassisted to stunning area
  - Difficult to transport and handle
  - Consumer perception

- Downers
  - "non-ambulatory"
    - Pathology
    - Fatigue
    - Lameness
  - Greatest handling risk
    - Stunning
    - Electric prod use
  - Not eligible for slaughter
    - Economic loss



- Body Condition Scoring
  - Lower the BSC the greater risk of mobility and handling issues
  - Consumer perception



Body Condition Score	Vertebrae at the middle of the back	Rear view (cross-section) of the hook bones	Side view of the line between the hook and pinbones	Cavity between tailhead and pinbone: Rear view	Cavity between tailhead and pinbone: Angled view
1 Severe underconditioning					
2 Frame obvious					
3 Frame and covering well balanced					
4 Frame not as visible as covering					
5 Severe overconditioning					

Figure 3: Body condition scores (Adapted from A.J. Edmondson, I.J. Lean, C.O. Weaver, T. Farver and G. Webster. 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72:68-78.)

- Transportation
  - Weather
  - Animal condition
    - Compromised health
    - Lameness
    - Calving
  - Type of animal
    - Horned
    - Bulls

- Bruising
  - Improper handling or poor equipment
  - Must be trimmed from carcass
  - Economic loss

Chemical Contamination

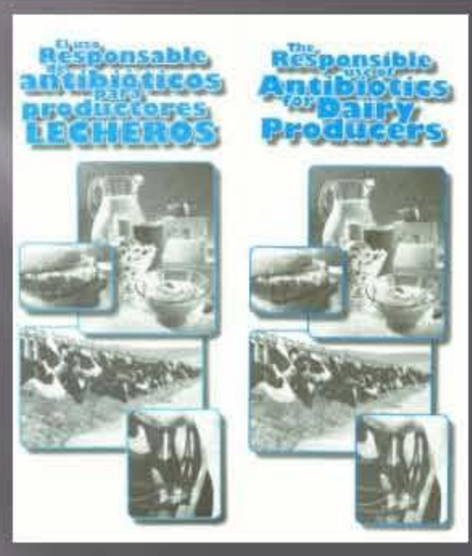
- Violative Drug Residues
- Repeat Residue Violations

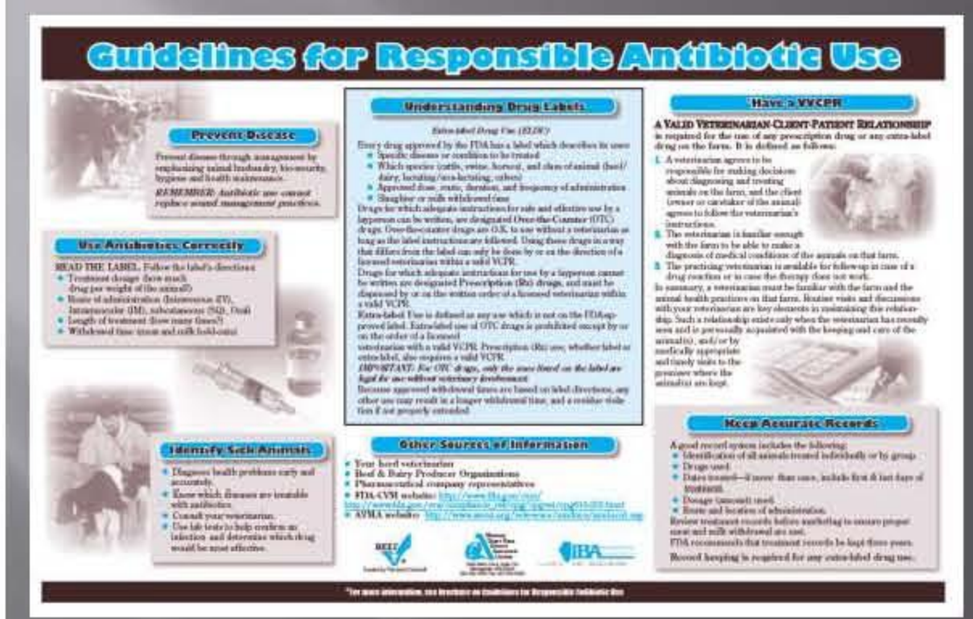
Addressing Chemical Residue

- Packers control this by:
  - Implementing a residue control program that is compliant with FSIS Directive 10,800.1 (July 12, 2007)
  - Monitor incoming cattle suppliers
  - Cross referencing the USDA Residue Violators List: http://www.fsis.usda.gov/PDF/Residue_EST.pdf
  - Communicating with the procurement department and cattle suppliers

Antibiotic Use Education

- Brochures
  - English & Spanish
- Posters
  - English & Spanish





Microbial Contamination

- *E. coli* O157:H7
- "Non O157s"
  - Shiga toxin producing *E. coli* (STEC)
  - *E. coli* serogroups O26, O103, O45, O111, O121 and O145
  - FSIS testing began June 2012
- Multi-Drug Resistant Salmonella

Addressing Microbial Contamination

- Keep cattle and facilities clean
- Maintain good herd health
- Don't use contaminated milk for feed
- Stay connected to evolving technologies and implement once developed.

Epitōpix

Humane Animal Handling

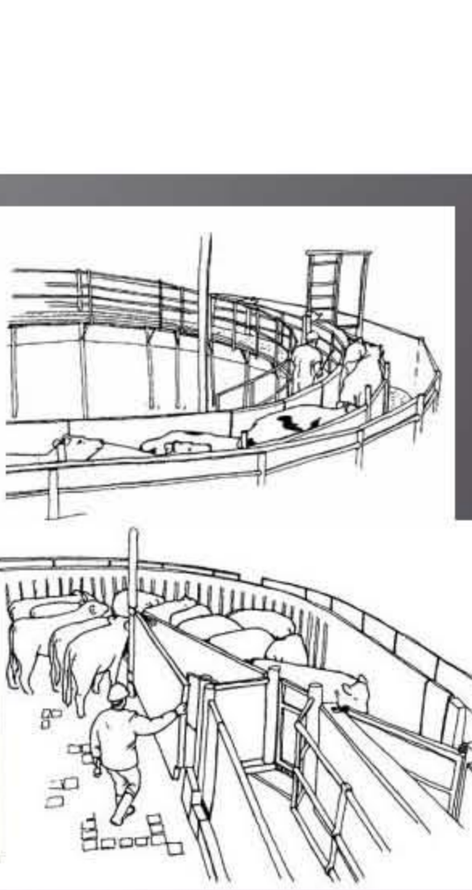
- At the farm
  - Animal handler practices
- Transportation
- At the plant
  - Animal condition
    - Downers
    - Lameness
- Results in:
  - safer working conditions
  - improves the quality of meat
  - decreases a significant financial loss

Plants Audit Daily

•Effective Stunning
•Insensibility
•Falls
•Vocalizations
•Electric Prod Use

Most critical is:

Willful Acts of Abuse



Egregious Acts

•Dragging a conscious, non-ambulatory animal

•Malicious driving of ambulatory livestock on top of one another either manually or with direct contact with motorized equipment

•Intentionally applying prods to sensitive parts

•Hitting or beating an animal

•Deliberate slamming of gates on livestock

•Animals frozen to the floor or sides of a trailer

AMI Foundation

Addressing Animal Handling

- Train
- Monitor
- Act
- Expect the same from your transporter

Animal Handling Resources

- Beef Quality Assurance resources
  - www.bqa.org
  - National Dairy BQA Producers Manual
  - Transportation Quality Assurance Program
  - Cattle Industry Guidelines for the Care and Handling of Cattle
- Minnesota Beef Council
- Temple Grandin, Ph.D.
  - www.grandin.com
  - Guidelines for auditing welfare

Dairy Producers Meat the Challenge!

- If we work together to address the issues
  - Quality concerns
  - Chemical contamination
  - Microbial contamination
  - Humane animal handling
- We can insure that dairy cows continue to be a valuable meat resource!
- Dairy beef stays on the fork!

AMERICAN FOODS GROUP

DAIRY BEEF IS ON THE FORK!

Producers have met the challenge!

Now how do they maximize the value?

AMERICAN FOODS GROUP

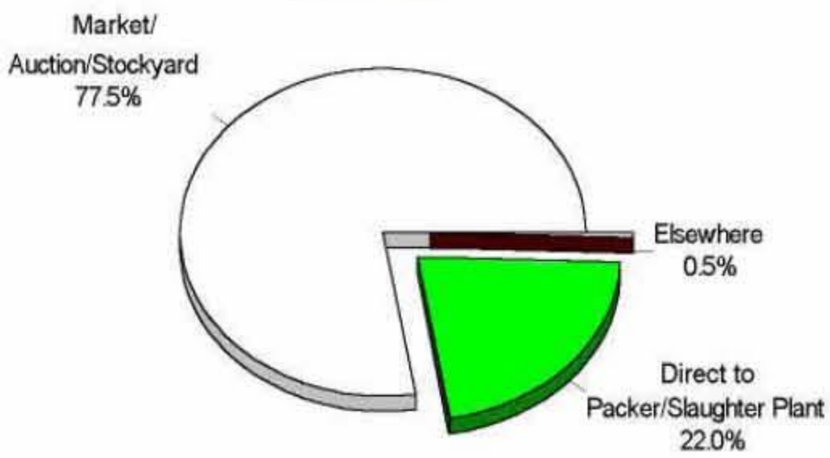
OPTIONS FOR MARKETING DAIRY COWS

Direct Marketing to Packer

Auction Markets/Stockyards

Dealers/Traders

Percent of Culled Dairy Cows Sold for Slaughter by Destination*



*For operations that culled dairy cows during 1995.

AMERICAN FOODS GROUP

HOW ARE COWS VALUED?

Carcass Yield
Red Meat Yield
Leanness
Subprimal Values

1.5 3 4.5

Figure 4: Examples of cows with body condition scores of 1.5 (A), 3 (B), and 4.5 (C)

informedfarmer.com

New Holland Auction, 3 Nov 08
By ANNE RUSSEK

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Euthanasia Guidelines for Cattle

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AVMA Guidelines for the Euthanasia of Animals: 2013 Edition

The newest version of the AVMA Guidelines on Euthanasia may be found at: https://www.avma.org/KB/Policies/Documents/euthanasia.pdf . The revised version is more comprehensive than previous editions and intended to be a "living document"; that is, to ensure the Guidelines remain as up-to-date as possible, interim revisions and other editorial corrections (e.g., typographical errors, updating of website addresses) will be made as necessary. A number will be given to each revision so that users will know if they are viewing the most recent version of the Guidelines.

Other changes relative to the 2012 Guidelines include the development of separate documents for Mass Depopulation and Humane Slaughter. The Panel determined that it was necessary to develop separate documents for these topics since the techniques applicable to mass depopulation and humane slaughter do not always fit the definition of euthanasia.

Introduction

In the following we have attempted to summarize the salient features of the AVMA Guidelines for euthanasia of cattle, including recent studies that did not make the deadline for inclusion in the revised version. One of these by Dr. JN Gilliam et al. is particularly noteworthy since the information presented in this paper represents a significant shift in current thinking on anatomical site selection for conducting euthanasia in cattle. A second study is that reported Dr. B. Wileman, et al. on firearm and munitions selection which supports observations from an earlier Canadian study indicating that the .22 LR loaded with a hollow point bullet lacks sufficient muzzle energy and physical characteristics to provide consistent results when used for the euthanasia of cattle.

In addition to proper application of euthanasia techniques, persons conducting euthanasia procedures need to understand the visual indicators of unconsciousness and the physical parameters that confirm death. Careful observation of these responses helps provide clues to the effectiveness of the euthanasia procedure and the possibility or likelihood of a return to sensibility. Proper interpretation of these responses is essential to assuring the welfare of animals that must undergo euthanasia.

There are many ways to induce death, but not all are humane nor would they fit the definition of euthanasia. Sometimes people resort to unapproved or unacceptable methods out of convenience

and/or a failure to understand that the particular method applied does not induce a humane death. It is imperative upon all who work with livestock to be prepared for situations that might require euthanasia of an animal. But, having the right equipment and a thorough understanding of the technique does not assure humane euthanasia. Too many animals still experience horrible deaths simply because of inertia and indecision. We discuss just a few of the causes of euthanasia delays. Once the decision is made, euthanasia should be conducted with as little stress to the animal as possible. This can be challenging as well as dangerous in some venues requiring tranquilization of some animals.

We conclude with a brief discussion of options for carcass disposal. This is no small concern as options for the disposal of carcasses have decreased markedly in many areas.

AVMA Guidelines on Euthanasia

Euthanasia means a "good death" whereby the methods applied to cause death induce an immediate loss of consciousness followed by cardiac and respiratory arrest and death without a return to consciousness. In the updated version of the AVMA Guidelines, euthanasia techniques are classified as 1) Acceptable, 2) Acceptable with Conditions, 3) Adjunctive, and 4) Unacceptable. Methods deemed "Acceptable" are those that consistently produce a humane death when used as the sole means of euthanasia. Those methods classified as "Acceptable with Conditions" are those that require certain conditions to be met in order to consistently produce a humane death. For example, techniques in this latter category might have greater potential for human error or injury and/or may require a secondary (adjunctive) step to ensure death. Although the "with conditions" qualifier suggests that these methods are less humane or not as suitable as those listed as "Acceptable", in fact they are considered to be equivalent to those listed under the "Acceptable" category.

Methods classified as "Adjunctive" are those that should not be used as the sole method of euthanasia; rather they are to be used in conjunction with others to ensure death in animals previously rendered unconscious. And finally, methods classified as "Unacceptable" are those that are considered to be inhumane under any conditions.

Methods of Euthanasia in Adult Cattle

Methods recognized as appropriate for euthanasia of cattle are: 1) barbiturates and barbituric acid derivatives ("Acceptable"), gunshot and penetrating captive bolt ("Acceptable with Conditions"). Penetrating and non-penetrating captive bolt are suitable for euthanasia of calves. Whether used in mature animals or in calves penetrating captive bolt requires an "Adjunctive" method to assure death. These are described in greater detail below.

"Acceptable" Methods

Barbiturates and barbituric acid derivatives—Barbiturates are preferred by some because of their rapid action and ability to induce a smooth transition from consciousness to unconsciousness and death. Drawbacks to the use of these agents for euthanasia include: cost, the need for restraint to deliver the drug, necessity to maintain a careful accounting of amounts used,

requirements that these agents be administered only by a veterinarian or personnel who are registered with the US Drug Enforcement Administration and finally, residues that limit carcass disposal options.

A question that frequently arises is: *"What happens to the fetus in pregnant animals euthanized by an overdose of pentobarbital"*? Research and clinical observation shows that barbiturates readily cross the placenta resulting in fetal depression; however death of the dam precedes death of the fetus by as much as 20-25 minutes. Fetal welfare is preserved by the fact that while *in utero,* the fetus is maintained in sleep-like state of unconscious. On the other hand, if removed from the uterus prior to 20-25 minutes post death of the dam, the fetus may regain consciousness. In cases involving euthanasia, any fetus removed from uterus prior to the amount of time required to cause death should be carefully observed for evidence of life and immediately euthanized if there is any doubt.

<u>"Conditionally Acceptable" Methods</u>

"Free Bullet" from Gunshot A 2008 study by Fulwider found that gunshot is the most common method used for on-farm euthanasia of cattle. Death by means of a "free bullet" is caused by massive destruction of brain tissue. Despite its popularity and effectiveness for the purpose of euthanasia, those who are less familiar with firearms often find gunshot violent and objectionable. However, as stated in a previous edition of the Guidelines:

"Properly applied, "euthanasia by either gunshot or penetrating captive bolt, causes less fear and anxiety and induces a more rapid, painless, and humane death than can be achieved by most other methods."

Penetrating captive bolt is also used for euthanasia of mature cattle in field situations. Unlike euthanasia with firearms, once the animal is rendered unconscious, an adjunctive method to insure death must be applied. Styles of penetrating captive bolt include an in-line (cylindrical) and pistol grip (resembling a handgun) versions. Pneumatic captive bolt guns (air powered) are limited to use in slaughter plant environments. Models using gunpowder charges are more often used in farm environments. Depending upon model, the bolt may automatically retract or require manual placement back into the barrel through the muzzle. Accurate placement of the captive bolt over the ideal anatomical site, energy (i.e. bolt velocity) and depth of penetration of the bolt determine effectiveness of the device to cause a loss of consciousness and death. Bolt velocity is dependent on maintenance, in particular cleaning and storage of the cartridge charges. Captive bolt guns should be cleaned regularly using the same or similar solvents used in the cleaning of firearms. Powder charges for the captive bolt should be stored in air tight containers to prevent damage from moisture in hot and humid conditions.

Non-penetrating captive bolt is not recommended for euthanasia of adult cattle. On the other hand, non-penetrating captive bolt is appropriate for euthanasia of calves when followed by the use of an adjunctive (secondary step) method to assure death.

<u>Research on Firearm Use for Euthanasia of Cattle</u>

Although the .22 LR is a popular caliber of firearm, results of a Canadian study suggest that it may not be the best choice for euthanasia of adult cattle because of poor penetration, deflection and fragmentation of the bullet. Standard and high velocity bullets fired from a .22 caliber rifle at a range of 25 meters (82 feet) failed to penetrate skulls of steers and heifers studied. These observations are corroborated by the results of a Kansas State University study by Wileman et al, designed to evaluate the characteristics of bullet penetration and brain tissue destruction using different calibers of firearms. In this study, researchers assigned disembodied heads of feedlot cattle to one of seven treatments: **1) *.22 LR with a solid point bullet*** (160 ft. lbs. or 217 Joules), **2) *.22 LR with a hollow point bullet*** (160 ft. lbs. or 217 Joules), **3) *.223 rifle*** (1183 ft. lbs. or 1604 Joules), **4) *9 mm handgun*** (316 ft. lbs. or 428 Joules), **5) *.45 caliber handgun*** (551 ft. lbs. or 747 Joules), **6) *12 gauge shotgun with # 4 shot*** (1769 ft. lbs. or 2398 Joules) and **7) *12 gauge loaded with a 1 oz. slug*** (4095 ft. lbs. or 5552 Joules). Cadaver skulls were shot from a fixed distance of 3 meters (approximately 10 feet). The anatomical site used was on the intersection of two lines each drawn from the medial canthus of the eye to the base of the opposite ear with the firearm directed toward the foramen magnum. Damage to the brain was determined by computed tomography (CT) using serial coronal scans at 3 mm intervals which were reconstructed at 1.5 mm intervals.

Results demonstrated that the .22 LR hollow point bullet had the poorest depth of penetration (107.5 mm) compared with other treatment groups which had a penetration depths of 150 mm. Only 33% of the 9 mm bullets caused damage to brain tissues sufficient to cause death. Greatest destruction of brain tissue occurred with the 12 gauge shotgun with #4 shot and the 1 oz. slug. Researchers concluded that the .22 LR with a hollow point bullet and the 9mm pistol could <u>not</u> be recommended based on this study.

A couple of points worthy of mention in regard to the above studies; first, when gunshot is used for the purposes of euthanasia, whenever possible the firearm should be held perpendicular to the skull and at a distance of no more than 2 to 3 feet away from the intended target. Reasons for these recommendations are to avoid ricochet and to take full advantage of the bullet's maximum muzzle energy. Obviously, this is not possible for an animal that is standing or mobile which is frequently the circumstance in feedlot conditions. In the studies cited above the distance of the shooters from their targets were 25 and 3 meters for the Canadian and US studies, respectively. As the distance away from the target increases so do the challenges for accurate shot placement, potential for ricochet and ability to maintain sufficient muzzle energy particularly when lower caliber firearms are used.

<u>Recommendations on Firearms for Euthanasia</u>

Handguns Handguns or pistols are short-barreled firearms that may be fired with one hand. For the purposes of euthanasia, handguns are limited to close-range shooting (within 1 to 2 feet or 30 to 60 cm) of the intended target. Calibers ranging from .32 to .45 are recommended for euthanasia of cattle. Solid-point lead bullets are recommended over hollow points because they are more likely traverse the skull. Hollow point bullets are designed to expand and fragment on impact with their targets which reduces the depth of penetration. The .22 caliber handgun is <u>not</u> recommended for routine euthanasia of adult cattle regardless of the type of bullet used, because

of the inability to consistently achieve desirable muzzle energies with standard commercial loads.

Rifles A rifle is a long barreled firearm that is usually fired from the shoulder. Unlike the barrel of a shotgun which has a smooth bore for shot shells, the bore of a rifle barrel contains a series of helical grooves (called rifling) that cause the bullet to spin as it travels through the barrel. Rifling imparts stability to the bullet and improves accuracy. For this reason, rifles are the preferred firearm for euthanasia when it is necessary to shoot from a distance. Rifles are capable of delivering bullets at much higher muzzle velocities and energies and are therefore not the ideal choice for euthanasia of animals in indoor or short range conditions. General recommendations on rifle selection for use in euthanasia of cattle include; .22 magnum, .223, .243, .270 and .308 and others.

Shotguns Shotguns loaded with birdshot (lead or steel BBs) or slugs (solid lead projectiles specifically designed for shotguns) are appropriate from a distance of 1 to 2 yards (.9 to 1.8 meters). Although all shotguns are lethal at close range, the preferred gauges for euthanasia of mature cattle are 20, 16, or 12. Number 6 or larger birdshot or shotgun slugs are the best choices for euthanasia of cattle. Birdshot begins to disperse as it leaves the end of the gun barrel; however, if the operator stays within short range of the intended anatomic site, the birdshot will strike the skull as a compact bolus or mass of BBs with ballistic characteristics on impact and entry that are similar to a solid lead bullet. At close range penetration of the skull is assured with massive destruction of brain tissue from the dispersion of birdshot into the brain that results in immediate loss of consciousness and rapid death.

Figure 1. Shotguns are a good choice for euthanasia using a firearm. A 20, 16 or 12 gauge are recommended for euthanasia of adult bovines. One may use birdshot (number 6 or larger BBs, or slugs.

One advantage of euthanasia using a shotgun is that within close range and when properly directed, birdshot has sufficient energy to penetrate the skull, but is unlikely to exit the skull. In the case of a free bullet or shotgun slug there is always the possibility of the bullet or slug exiting the skull creating an injury risk for the operator or by-standers. For safety reasons it is important that the muzzle of a shotgun (or any other firearm) never be held directly against the animal's head. Discharge of the firearm results in the development of enormous pressure within the barrel that can result in explosion of the barrel and potential for injury of the operator and by-standers if the muzzle end is obstructed or blocked.

Captive Bolt

Penetrating captive bolt In general, captive bolt guns, whether penetrating or non-penetrating, induce immediate loss of consciousness, but death is not always assured with the use of this device alone. Therefore, an adjunctive method such as a second shot, exsanguination, pithing or the intravenous injection of a saturated solution of potassium chloride (KCl) is recommended to ensure death when penetrating captive bolt is used. A newer version of penetrating captive bolt has emerged in recent years. This device is equipped with an extended bolt with sufficient length and cartridge power to increase damage to the brain including the brainstem. If studies prove this to be an effective 1-step euthanasia method, it will eliminate the need for an adjunctive method. Unlike techniques described for gunshot, the animal must be restrained for accurate placement of the captive bolt. And, unlike use of a firearm, proper use of the captive bolt requires that the muzzle of the device be held firmly against the animal's head. Once the animal is restrained, discharge of the captive bolt should occur with little or no delay so that animal distress is minimized. Adjunctive methods should be implemented as soon as the animal is rendered unconscious to avoid a possible return to sensibility. Thus, when conducting euthanasia by captive bolt, pre-planning and preparation is necessary to achieve the desired results.

Visual indicators that an animal has been rendered unconscious from captive bolt or gunshot include the following: immediate collapse; brief tetanic spasms followed by uncoordinated hind limb movements; immediate and sustained cessation of rhythmic breathing; lack of coordinated attempts to rise; absence of vocalization; glazed or glassy appearance to the eyes; centralized eye position with a dilated pupil; and absence of eye reflexes. Nervous system control of the blink or corneal reflex is located in the brain stem; therefore, the presence of a corneal reflex is highly suggestive that an animal is still conscious.

Anatomical Landmarks for Euthanasia of Cattle

The objective in euthanasia is to cause sufficient damage to the brain to result in immediate loss of consciousness and death. Accomplishment of this objective requires the accurate delivery of a bullet or captive bolt at an anatomical site that is most likely to cause damage to the brainstem. In the past, most recommendations suggested that the ideal site was on the intersection of two lines each drawn from the medial canthus of the eye to the base of the opposite horn or top of the ear in polled cattle.

Figure 2. Cadaver skull form an adult Holstein cow shot with a penetrating captive bolt. The shot was placed on the intersection of 2 lines each from the medial canthus (inside corner) of the eye to the opposite horn or top of the opposite ear (black arrow). Note that the bolt failed to enter the cranial vault (white arrow). Path of the bolt is rostral to the brain (Gilliam et al., 2012).

As early as 2008, Gilliam and others suggested that this site was in fact too rostral (i.e. toward the nasal region or muzzle) and unlikely to damage the brainstem (See Figure 1). In order to confirm this observation, Gilliam instituted a study to evaluate the likelihood of brainstem damage using penetrating captive bolt at two anatomical locations. Cadaver skulls from 15 cattle were divided into one of two groups. Group 1 was shot with the penetrating captive bolt on the intersection of two lines each drawn from the medial canthus of the eye to the opposite horn or top of the opposite ear. Group 2 was shot at the intersection of two lines each drawn from the lateral canthus of the eye to the opposite horn or top of the opposite ear. The actual tract (or path) of the bolt for each respective location was determined by computed tomography and physical observation of the brain and brainstem. Evaluation of the skulls from Group 1 demonstrated that the bolt failed to make contact with the brainstem in all skulls studied (See Figure 2). In Group 2, the bolt was observed to cause significant damage to the brainstem in 6 of 8 skulls studied (See Figure 3). These results, although preliminary, indicate that the higher anatomical site improves the likelihood of causing damage to the brainstem. However, these data also suggest that some adjustment of this site is still necessary to achieve consistent results. This study is continuing with plans to assess age and breed differences for determination of the best anatomical site for conducting euthanasia in cattle.

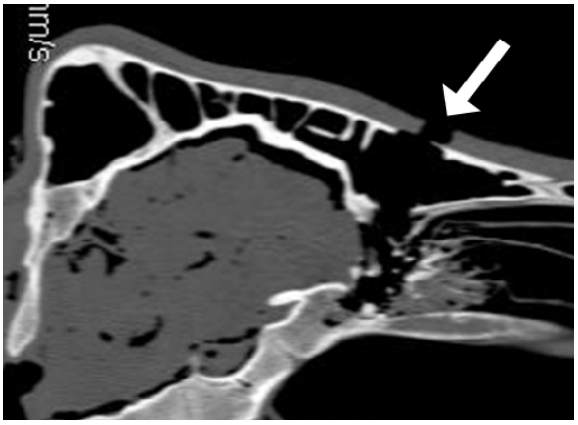


Figure 4. Computed tomography image of a bovine skull shot with a penetrating captive bolt showing the bolt path (white arrow) too far rostral to disrupt the brainstem (Gilliam et al. 2012).

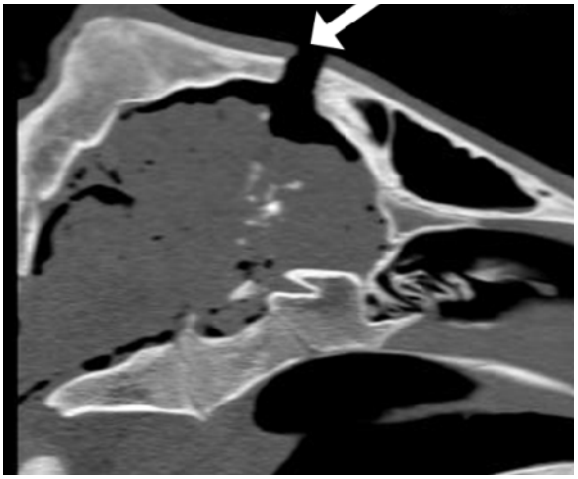


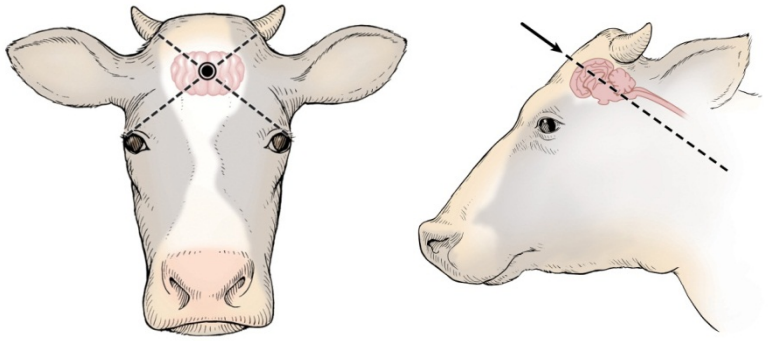
Figure 3. Computed tomography image of a bovine skull shot with a penetrating captive bolt showing the bolt path (white arrow) disrupting both the cerebral cortex and brainstem (i.e. bolt path is indicated by bone fragments pushed into the brain, Gilliam et al 2012).

Anatomic landmarks for use of the penetrating captive bolt and gunshot Based upon current information in cattle, we suggest that the point of entry of the projectile should be at (or slightly above) the intersection of two imaginary lines, each drawn from the outside corner (lateral canthus) of the eye to the center of the base of the opposite horn. If a firearm is used it should be used within 3 feet of the target when possible and positioned so that the muzzle is perpendicular to the skull to avoid ricochet. When using penetrating captive bolt, operators are advised to restrain the head so that the captive bolt may be held flush with the skull.

In all cases, proper positioning of the firearm or penetrating captive bolt is necessary to achieve the desired results. As suggested earlier, persons using captive bolt are advised to prepare for the

application of adjunctive methods to assure death as soon as possible following confirmation that the animal is unconscious. It is also important to consider positioning of the captive bolt device. Directing the bolt toward the foramen magnum will likely improve results particularly when placement of the device is slightly rostral.

Figure 4—Anatomic site for gunshot or placement of a captive bolt and desired path of the projectile in bovids.



Poll Stunning

Many people assume the poll (the highest point on the skull) is a proper site for conducting euthanasia procedures with either gunshot or penetrating captive bolt. In fact, this site is not advised since studies indicate that the depth of concussion in this region is less than that observed with frontal sites. Furthermore, research indicates that the use of penetrating captive bolt at the poll is more prone to operator error and misdirection of the bolt into the spinal cord instead of the brain. Conversely, for large bulls and water buffalo use of the frontal site is not always effective because of the thickness of the hide and skull in this region. Use of the poll position can be effective if the appropriate captive bolt gun is used and when the muzzle is directed so that the discharged bolt will enter the brain; but this site is not recommended for routine use.

Unacceptable Methods

The methods of euthanasia deemed unacceptable include: 1) manually applied blunt force trauma (as with a large hammer), 2) injection of chemical agents or other substances not specifically designed or labeled for euthanasia (i.e. disinfectants, cleaning solutions, etc.), 3) air injection into the vein, 4) electrocution as with a 120 volt electrical cord, 5) drowning, 6) exsanguination of conscious animals, and 7) deep tranquilization as with xylazine or other alpha-2 agonist followed by potassium chloride or magnesium sulfate. While some have been forced out of desperation to resort to one or more of these methods, readers are strongly advised against their use. Several of these methods are known to result in a less than humane death and for others the level of pain or distress associated with these methods is unknown. For example, use of xylazine to create a deep state of tranquilization followed by the rapid administration of KCl is used by some veterinarians. The position of the AVMA is as that stated in Goodman and Gilman's Pharmacological Basis of Therapeutics, 11th Edition: "Although large doses of alpha-2 agonists can produce a state resembling general anesthesia, they are recognized as being unreliable for

that purpose." Therefore, until such time as we have better information on this method in terms of its ability to cause a humane death, it is best to utilize alternate techniques.

Confirmation of Death

Regardless of method used for conducting euthanasia procedures it is important to confirm death. It is sometimes more easily said than done. However, the most reliable criteria include lack of pulse, breathing, corneal reflex and response to firm toe pinch, inability to hear respiratory sounds and heart beat by use of a stethoscope, graying of the mucous membranes, and rigor mortis. None of these signs alone, with exception of rigor mortis, confirms death.

The Impediments to Timely Euthanasia

No one enjoys the task of euthanasia or really wants to do it. This is especially so for a livestock owner faced with the task of euthanizing his/her own animal. Employees face similar problems in conducting these procedures and for the same reasons. Some develop close attachments for the animals within their care. The physical methods of gunshot and penetrating captive bolt are inherently violent. While this is a significant deterrent in itself; in addition, many people are unfamiliar with the proper use of firearms, let alone captive bolt. Sometimes the question that prevents moving forward with timely euthanasia is related to an uncertain prognosis. Diseased and/or injured animals often exhibit conflicting signs; it's not always a black or white decision as to whether or not euthanasia is indicated. The opportunity to error on the side of waiting too long looms large.

The consequence of early euthanasia is largely economic and delaying it prolongs animal suffering. Veterinarians play a key role in assisting folks with these decisions and should be consulted whenever there are doubts as to whether euthanasia is warranted. When necessary or desired, veterinarians can intervene and relieve their clients of the burden of conducting the task on an animal to which they are emotionally attached. Euthanasia decisions can be complicated and some will undoubtedly be haunted by those lingering questions for which some might find consolation in the words of Dr. Bernard Rollin, Professor of Philosophy and Bioethics at Colorado State University, *"Better a week too early than a day too late"*.

Considerations for Conducting the Procedure

Persons conducting euthanasia procedures should attempt to minimize animal distress. If animals are accustomed to human contact the presence of a familiar person may be reassuring and reduce anxiety. For animals that are not accustomed to human contact, gunshot may be the best option for euthanasia simply because it can be delivered with the least amount of human contact. In some cases tranquilization may be necessary to quiet a frightened or anxious animal.

Cattle should be approached quietly and restrained only as necessary to properly conduct the procedure. If the animal is ambulatory and able to be moved without causing distress, discomfort or pain, it may be relocated to an area where the carcass may be more easily reached by removal equipment. Dragging of non-ambulatory animals is unacceptable. In cases where movement of a

down animal would increase distress or animal suffering, the animal should be euthanized first, and then moved following confirmation of death.

Euthanasia of Injured or Recumbent Cattle on Enclosed Trailers

Not all cattle requiring euthanasia are found in the farm or ranch setting. Some are the consequence of livestock truck roll-over accidents or cattle injured in the process of hauling to a market or packing plant. Whenever an animal is down and unable to voluntarily walk off of a trailer, it may become necessary to euthanize the animal prior to removal. Since entering the trailer with a fractious animal (dairy bull or beef animal) might put a person at considerable risk, and gunshot is unsafe and possibly restricted by local ordinances, tranquilization of the animal is necessary. This can be accomplished by a veterinarian with a medicated dart from either a pistol or rifle, or by use of a "pole syringe" of sufficient length to deliver the tranquilizer from across a barrier between the operator and the agitated animal. Xylazine dosed at 0.3 to 0.5 mg/lb. (3 to 5 CC of 100mg/Ml /1000 lbs.) is usually sufficient to render the animal safe to approach.

Readers are cautioned that although this is a larger dose than that one would normally use, the combination of administering the drug to an anxious animal plus delivery via a dart or pole syringe makes the end result less predictable. Following administration of the xylazine, leave the animal undisturbed for the 15 to 20 minutes required for the xylazine to take full effect. Once the animal is sufficiently tranquilized it may be approached for application of the penetrating captive bolt with adjunctive procedures to ensure death. The primary concerns in these situations are human, animal and food safety.

Carcass Disposal

Euthanasia presents another issue that people frequently fail to consider – disposal of the carcass. In North America, there are plenty of coyotes, buzzards and other scavenging animals willing to assist with carcass removal. This seems a natural way to dispose of an animal carcass; it serves the purpose of disposing of the carcass and provides food for the scavengers. This practice may be acceptable on large acreages, especially those without nearby neighbors and areas containing upland woods and brush. However, this natural method isn't permitted in most areas, and some scavengers become predators when carcasses are less available. This places newborn calves and other animals weakened by disease or other maladies at risk of predation. Most cattle producers are well aware that coyotes can take a significant toll on newborns. In dairy operations, calves may be attacked at birth or later when confined to a small pen or hutch. In either situation, they are easy prey for a coyote. Furthermore, a proper method of carcass disposal is needed to prevent the spread of infectious and/or contagious disease. Finally, as described earlier, when barbiturates are used for euthanasia wildlife may be at risk from the consumption of carcasses with drug residue that may be deadly. Penalties for the accidental killing of endangered animals are severe and include incarceration as well as huge fines for persons convicted of the offense.

The problem is that socially, economically and environmentally acceptable methods of carcass disposal have become increasingly difficult to find. In the United States the disposal of animal carcasses is regulated by state and local laws that vary widely according to animal species. The

most common methods for disposal of animal carcasses are burial, composting, incineration and rendering. Less common methods would include landfills and tissue digestion.

Advances in analytical chemistry have led to increasingly sensitive assays for multiple drugs and antimicrobials. The ability of these technologies to identify residue at extremely low levels has also continued to increase the scrutiny of rendered product end users. Today, acceptable levels are no longer acceptable, for many if not most, only zero tolerance will do. The result is a rendering industry that is much less accepting of the carcasses of animals euthanized by barbiturates. Therefore, the first choice recommendation for carcass disposal of animals euthanized by pentobarbital overdose is incineration or cremation; but cost precludes this from being an economically viable consideration for carcass disposal in most of today's commercial farm operations. The next best option is burial of the animal sufficiently deep to avoid being exhumed by scavengers. This must be conducted in accordance with State and local laws to assure no contamination of ground water sources. When the ground is frozen, carcasses must be carefully covered and stored until such time as burial may be possible.

Composting is another means of carcass disposal that is becoming increasingly more common. Although studies are few, most report the persistence of barbiturate residues in composted material. For these reasons, the physical methods of gunshot and captive bolt are far more attractive for euthanasia of livestock. Even when adjunctive methods such as the rapid intravenous administration of potassium chloride are used to assure death there are no worries for rendering or composting. In short, while there are many within the veterinary profession that find gunshot and penetrating captive bolt violent methods and therefore less desirable, when they are properly conducted they are very humane, cost-effective and do not pose residue risks.

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How to Minimize the Impacts of Dystocia on the Health and Survival of Dairy Calves

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- **Take Home Messages**
- The most dramatic physiological changes occur during birth and death.
- Calves born without assistance experience hypoxia (low arterial blood oxygen), acidosis, and frequently hypothermia. Dystocia calves experience the same changes but to a greater degree.
- Dystocia has an immediate and prolonged effect on the health and productivity of calves.
- Perinatal mortality due to dystocia accounts for about half of all calf deaths through weaning and is not due to infectious disease.
- Simple interventions such as providing calves with mechanical breathing assistance, oxygen, colostrum, and a warm environment for the first few hours after birth can make the difference between life and death.
- Dystocia monitoring should be implemented on every dairy farm.
- Dystocia and subsequent perinatal mortality are major animal-welfare issues for the dairy industry.

Prevalence and effects of dystocia

Dystocia is defined as delayed or difficult parturition. There are three general categories of dystocia: fetal-maternal size mismatch (e.g. oversized calf or small maternal pelvis), fetal malpresentation (e.g. breech presentation), and maternal causes (e.g. hypocalcemia) (Arthur et al., 1989).

The prevalence and effects of dystocia can be reduced in three ways:

1. Prebreeding management: select sires for calving ease and dams for adequate pelvic size (dam selection has never been done in the dairy industry), breed heifers of recommended height and weight, and provide optimal nutrition during pregnancy.

2. Calving time: ensure that calving areas are comfortable and as stress free as possible, and provide assistance when needed using proper techniques and procedures.

3. Neonatal assistance: provide maternal and additional care as needed.

While all three of these methods are important, this paper focuses on neonatal assistance.

Most dairy farms likely don't know the impact of dystocia because it isn't well monitored. The first step in reducing dystocia and improving the outcome for calves is to implement a monitoring plan. The plan can be relatively simple. For instance, the first step would be recording the number of calvings using a three-point dystocia scale in which '1' is an unassisted birth, '2' is a relatively easy pull, and '3' is a hard pull or surgical extraction. Each calving should be assigned a dystocia score using the above criteria. In addition, record live and dead births, gender, time to stand, time to colostrum, and time to suckle. Specific interventions are discussed later in this paper and can also be recorded.

These basic measures can be used to evaluate the effect of dystocia and suggest interventions to decrease its impact. Evaluated over time, these measures can indicate whether or not management changes have reduced the occurrence and impact of dystocia. For instance, a comparison could be made between the incidence of dystocia and the number of stillbirths by the level of dystocia before and after the implementation of specific practices, such as using a new sire, worker training, or additional care provided to dystocial calves.

The prevalence of dystocia varies based on the type of cattle (beef versus dairy breeds), parity, and across studies. Data collected from U.S. dairy farms participating in the Dairy Herd Improvement Association indicated that 28.6% of primiparous (first-calf heifers) and 10.7% of multiparous cows experienced dystocia (Meyer et al., 2001). Other producer-collected data suggest a much lower rate or, more likely, reduced recognition of dystocia. These data indicate that only 4.6% of cows experienced reproductive problems, including dystocia (NAHMS 2007). A study involving three large U.S. dairies reported an overall dystocia rate of 36.6% (Lombard et al., 2007), with 48.8% of primiparous dams experiencing dystocia compared with 29.4% of multiparous dams. Holsteins, the predominant dairy breed in the United States, have a higher prevalence of dystocia than Jerseys or Jersey crosses (Dhakal et al., 2013). Regardless of breed, twins, bull calves, and heavier calves have an increased risk of experiencing dystocia (Johanson and Berger, 2003; Dhakal et al., 2013).

Multiple studies have demonstrated the adverse effect dystocia has on the survival, health, and production of calves and dams. (Lombard et al., 2007; Barrier et al., 2012 JDS; Barrier et al. 2012 TVJ; Tenhagen et al., 2007). A 13-month study evaluating 7,380 calvings on three Colorado Holstein dairy farms (Lombard et al., 2007) used a three-level dystocia scoring system with 1 indicating a normal, unassisted delivery; 2 indicating assistance by one person not using mechanical means; and 3 indicating that two or more

people were required, and mechanical or surgical assistance was required. In this study, the percentage of stillbirth calves (more correctly perinatal mortality) increased as the dystocia score increased. Only 3.2% of unassisted calves (score of 1) were stillborn compared with 8.4% of calves with a score of 2, and 37.2% of calves with a score of 3. Overall, 8.2% of calves were stillborn, which is similar to a review of perinatal mortality reported by Mee (WCDS, 2012).

Studies of both dairy and beef calves have also shown increased morbidity and mortality through the preweaning period for calves experiencing dystocia (Lombard et al., 2007; Wittum et al., 1994). Dystocial calves in the Lombard study had significantly increased odds of experiencing respiratory or digestive disease compared with calves born unassisted. A similar finding of increased odds for general morbidity was reported in beef calves experiencing dystocia in the Wittum study. Based on the findings from these studies, dystocia has both an immediate and prolonged effect on the health and survival of calves. Obviously, based on the frequency of occurrence and the impact, dystocia should be an area of great concern for the dairy industry.

To address the impact of dystocia on calves, it is helpful to understand the complex and dramatic physiological changes that must occur for calves to successfully adapt to extrauterine life.

Normal fetal physiology

During normal extrauterine circulation in calves, the right side of the heart receives deoxygenated (relatively low oxygen and high carbon dioxide) blood from the body and pumps it to the lungs where oxygen is taken up by hemoglobin in the red blood cells and carbon dioxide is removed. The oxygenated (relatively high oxygen and low carbon dioxide) blood then flows to the left side of the heart where it is pumped to the rest of the body. Blood pressure in the right side of the heart is lower than in the left side (Cunningham and Klein, 2007).

The lungs of the fetus *in utero* are not functional. Until birth, the placenta serves as ‘fetal lungs,’ providing gas exchange and acting as the source of nutrients. The placenta also eliminates waste products such as carbon dioxide from fetal blood. In turn, diseases or disorders affecting the placenta can have serious consequences for calves. Fetal blood circulation bypasses the lungs, for the most part, since blood is not oxygenated *in utero.* Fetal blood travels through two structures (referred to as fetal shunts) to bypass the lungs. The foramen ovale is a connection between the right and left atria of the heart and prevents some blood from being pushed through the lungs. The ductus arteriosis connects the pulmonary artery to the aorta and also shunts blood away from the pulmonary circulation (Kasari 1994).

During pregnancy and immediately after birth, calves have low blood oxygen levels and relatively high carbon dioxide levels compared with calves just a few hours old. Fetuses do fine at lower oxygen levels *in utero* because of the reduced oxygen consumption associated with low physical activity and living in a controlled environment in which the dam is responsible for providing nutrients and removing waste products.

Changes at parturition

Parturition results in dramatic physiological changes and has a negative impact on fetal oxygen concentration. As delivery progresses, uterine and abdominal contractions can impede or stop blood flow through the placenta. Bluel et al., (2008) showed that during delivery a fetus's blood oxygen saturation drops from about 20% to less than 5%. During the calving process, the fetus experiences neonatal asphyxia: low blood oxygen levels and areas of decreased blood flow, or ischemia. Hypoxia can progress to anoxia (no oxygen in the blood). Prolonged anoxia, such as occurs during continuous uterine contractions, will result in fetal death within six minutes.

After delivery, the calf must begin breathing by inflating the lungs and initiating gas exchange. Lung expansion reduces blood pressure on the right side of the heart, causing a reversal of blood flow through fetal shunts, and functionally occludes the foramen ovale, usually within 5 to 20 minutes after birth. Due to respiration, oxygen tension in the blood going to the left side of the heart increases, causing the ductus arteriosus to close, usually within 5 minutes after birth (Kasari 1994). If the foramen ovale or ductus arteriosis do not close normally, the resulting turbulent blood flow may be detected as a heart murmur.

Increased blood levels of carbon dioxide result in a respiratory acidosis and play a critical role in stimulating respiration. During dystocia, a more pronounced respiratory acidosis may occur. In addition to respiratory acidosis, the reduced oxygen content of the blood leads to anaerobic metabolism within tissues, resulting in a metabolic acidosis (lactic acidosis). The major clinical effect of acidosis is central nervous system depression, sometimes referred to as 'weak calf syndrome' or 'dummy calf syndrome'. Maximizing lung function is key to resolving respiratory acidosis in newborn calves. Once the lungs are expanded, carbon dioxide is quickly removed via respiration. Resolution of the metabolic acidosis usually occurs within 2 hours of birth, while respiratory acidosis may persist for 24 to 48 hours (Ravary-Plumioën, RMV 2009).

After parturition, the neonate moves from the controlled environment of the uterus to the ambient environment, which always results in heat loss. Minimizing heat loss by drying the hair coat and placing calves in a protected and warmed environment will increase the calves' body temperature. Calves also begin to generate body heat after birth. Heat is generated by three mechanisms: shivering thermogenesis, non-shivering thermogenesis, and physical activity. Shivering thermogenesis involves involuntary, periodic skeletal

muscle contractions, while nonshivering thermogenesis involves the metabolism of brown adipose tissue (brown fat). Physical activity is the voluntary movements of skeletal muscles and is responsible for the greatest heat generation (Carstens, 1994). All of these mechanisms require good blood oxygen levels; hypoxemic calves have reduced ability to generate heat.

Effects of dystocia

Dystocia results in a more severe acidosis than a normal, unassisted birth due to the increased time of hypoxia and anoxia during parturition. The longer calves are in the transition between the uterine and extrauterine environment, the greater the probability of anoxia, resulting in a more severe acidosis. The acidosis starts a cascade of events that make the successful transition to extrauterine life much more difficult. Dystocial calves frequently have a depressed central nervous system, which reduces the stimulation for respiration. This depression also results in decreased physical activity and might prevent calves from standing or taking longer than normal to stand. In addition, decreased physical activity and reduced shivering results in more heat loss and hypothermia. In this case, suckling and the consumption of colostrum may not occur and, if it does, calves may not efficiently absorb the immunoglobulins necessary to protect against disease. Hypothermia and the lack of activity result in not only a failure to resolve the acidosis, but it commonly gets more severe and these calves frequently die (Kasari, 1994).

Calving assistance

Assistance should always be provided by a trained, competent person. Information on diagnosing and treating dystocia has been published elsewhere (Roberts, 1986). In addition to knowing the techniques involved in delivery, it is important to implement the proper use of hygienic procedures, lubrication, chains, and other extraction equipment, which should result in a successful outcome for calves and dams. Farms should have guidelines available that provide specific instructions on when and how to proceed during a dystocia event. In addition, dystocia training for employees can help decrease the effects of dystocia. Improper or aggressive methods used by untrained personnel are likely to cause physical harm to calves, including fractures and crushing injuries (Nagy, 2009). In a study by Schuenemann et al., (2011) employees underwent a comprehensive educational program designed to improve calving management, practices of calving personnel, and communication within the farm team. One herd was evaluated for stillbirths before and after the training; the stillbirth percentage on this farm dropped from 15.5 to 6.5% after training, representing a 60% decrease in stillbirths. Similar reductions due to employee training have been observed by the authors.

In order to know when and when not to intervene, the calving process must be thoroughly understood. Although the normal calving process is classified into three stages, the

process is continuous and proceeds gradually from one stage to the next. Stage 1 is characterized by cervical dilation and uterine contractions that are usually not evident as abdominal contractions. During this stage, cattle might be restless/off feed because of the discomfort caused by uterine contractions. Stage 1 usually lasts 2 to 6 hours but may be longer in heifers. During stage 2, uterine contractions continue and abdominal contractions become evident. Stage 2 ends in the delivery of the fetus(es) and usually takes less than 2 hours for mature cows but up to 4 hours for heifers. In stage 3, the fetal membranes (placenta) are expelled as the uterus continues to contract. The duration of stage 3 can be minutes, even days if the placenta is retained (Arthur et al., 1989).

Frequently observing cows close to calving is key in determining how labor is progressing, how much time has elapsed since labor began, and whether or not intervention is necessary. About half of U.S. dairy producers reported observing cattle close to calving about every 3 hours during the day but only about every 5 hours during the night (NAHMS, 2007). When calving was imminent and the heifer or cow was restless/off feed but not straining, about half of producers would examine heifers and cows within 3 to 5 hours; however, more than one-fourth of producers waited 7 or more hours before examining cattle in labor. Once heifers or cows began straining, almost 90% of producers examined the animals within 3 hours if labor was not progressing. Producers that call a veterinarian for assistance should consider the time it takes the veterinarian to arrive on site to avoid increasing the time to delivery and the possibility of a dystocia related stillbirth. The results of the NAHMS study indicate that many producers should observe cattle in labor more frequently and potentially intervene earlier in the calving process.

Neonate assistance

Based on the many physiological changes that occur during and after birth, most neonates, especially those experiencing dystocia, can benefit from relatively simple interventions. The three goals of intervention are to:

1) Stimulate respiration

2) Maintain body temperature (thermoregulation)

3) Increase blood volume via colostrum

Stimulate Respiration

As mentioned earlier, calves are born with increased levels of carbon dioxide in their blood, which stimulate respiration; however, sometimes calves still need assistance breathing. To help calves breathe, mucus in the upper airway should be removed via suction or positive pressure ventilation. Some farms suspend calves from their rear legs

immediately after birth to help clear fluid from the upper airway and lungs. Research has shown that calves delivered by caesarean section have improved gas exchange and acidosis correction when they are suspended for 90 seconds or less or placed in sternal recumbeny compared with calves placed in lateral recumbency (Uysterpruyst et al., 2002). Although suspending newly born calves might be beneficial if done for a very short period, research has shown that most of the expelled fluids originate in the calves' abomasum, not in the lungs. Additionally, the weight of the digestive tract on the diaphragm when calves are suspended makes it more difficult for calves to breathe. Rather than suspending calves upside down, we prefer to place calves in sternal recumbency immediately after birth.

Lungs must inflate with air and expand in order for respiration to occur. The initial expansion of the lungs is similar to blowing up a balloon; expansion is difficult initially but gets easier as more air enters the lungs. Increased blood levels of carbon dioxide and the additional stimuli of the extrauterine environment, including temperature changes, promotes respiration in calves. Additional stimulation and respiration assistance can be performed in multiple ways, including stimulating the nostril with a piece of straw or similar material, vigorously rubbing the calves, pouring cold water on the calves' head or in their ears, using an Ambu bag or similar device, or endotracheal tube for positive pressure ventilation, drugs such as doxopram, and the administration of oxygen. We recommend stimulation, positive pressure ventilation, and the administration of oxygen, for calves experiencing dystocia. We don't recommend pouring cold water on newborn calves because of hypothermia concerns.

Positive pressure ventilation forces air through the upper airway or trachea and into the lungs using mechanical means. Devices such as the Ambu bag have pressure relief valves that prevent over inflation and damage to the lungs. When using an Ambu bag with a mask for positive pressure ventilation, the esophagus must be occluded to prevent inflation of the abomasum (Kasari 1994). Occluding the esophagus can usually be accomplished by extending the neck and applying pressure around the trachea. Some calves may need only a few "mechanical" breaths to inflate their lungs, while others may need more prolonged assistance. Endotracheal tubes can also be used, but they frequently require a laryngoscope to place within the trachea and are not as simple and convenient to use as the Ambu bag with a mask.

The direct administration of oxygen via nasal insufflation can be accomplished using a small rubber catheter placed in the nose or integrated into the ventilation provided with the Ambu bag. The recommended oxygen flow rate varies, but 2 to 4 L/min is probably adequate for most calves (Bleul 2008; Nagy 2009). The length of time that oxygen should be administered is based on the response of the individual calf. If the calf appears to be doing well, oxygen administration can be discontinued and the calf monitored to determine if oxygen therapy should be reinstituted.

Thermoregulation

Newborn calves regulate their body temperature (i.e. generate heat) by the catabolism of brown fat and by activity. Hypothermia, or body temperatures below 98.6°F, occurs in up to 25% of calves at birth (Mee, 2008). Calves that can thermoregulate shouldn't have a body temperature less than 101°F. Heat loss in calves occurs in multiple ways. In most instances, the ambient temperature is lower than the calves' body temperature, resulting in heat loss through convection. Calves will also lose body heat when lying on cold surfaces via conduction. The other common form of heat loss occurs via evaporation from wet calves. Dystocial calves have increased heat loss and lower body temperatures due to their acidosis and decreased activity. It is relatively easy to assist calves in thermoregulation by drying them immediately after birth. Drying calves not only stimulates respiration but also reduces evaporative heat losses. Providing straw or other bedding also reduces conductive heat loss. Increasing the ambient temperature using a heater, providing heat via a hot water bottle, or immersing the calves in hot water helps prevent and treat hypothermia. Calves may need heat sources for up to 24 hours after birth.

Administer Colostrum

Once calves are breathing normally, administering high quality colostrum is one of the most important practices to increase calves' survival and productivity. There are numerous papers on the importance of colostrum administration for preventing failure of passive transfer and subsequent health and productivity of heifers. Colostrum also provides essential fluids that are absorbed by the calves, increasing blood volume, thereby improving circulation and resolution of acidosis. Colostrum is also an important source of energy. This energy and the fact that colostrum is given to calves at 100F helps calves regulate their body temperature.

Conclusion

There have been large field studies evaluating the negative effects of dystocia. These effects are numerous, consistent, and have a negative impact on the health and welfare of calves and dams. Since dystocia is associated with 50% of preweaned calf losses, every dairy should implement a dystocia monitoring program and employ management practices that limit the occurrence and impact of dystocia.

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Digital dermatitis in cattle

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Digital dermatitis is the number 1 cause of lameness in dairy cattle and rapidly emerging as a major cause of lameness in feedlot cattle. Predisposing factors include: purchasing or raising replacements off-site, large herd size, muddy corrals (wet manure covered feet),use of a hoof trimmer that trims feet on other dairies, failure to wash or disinfect hoof care equipment between farms, facilities with grooved concrete, use of a footbath, herds with Holstein cows versus other breeds and more. Organisms from the genus *Treponema sp*. are consistently isolated from lesions of digital dermatitis; but most researchers are reluctant to believe that these organisms are acting alone in the pathogenesis of this disease. Treatment and control remains limited to topical spray treatment, footbaths and topical antibiotics with or without a bandage.

Digital Dermatitis (Footwarts, Hairy Heel Warts, etc.)

Discovery of the Disease

Italian researchers, Drs. Cheli and Mortellaro are credited with being the first to describe this disease in Italy in 1974. However, a report by Lindley in 1974, describing a similar raised wart-like lesion in bulls he termed “malignant verrucae” suggests that digital dermatitis was quite likely present during this time in the US as well. A better known report from outbreaks of DD in New York dairy herds was published in 1980. Since that time DD has become worldwide in occurrence.

Multiple Names

Digital dermatitis is known by a number of terms: footwarts, hairy heel warts, digital warts, strawberry foot, raspberry heel, verrucose dermatitis, Mortellaro or Mortellaro’s disease (after Dr. Carlo Mortellaro), papillomatous digital dermatitis (PDD) or simply, digital dermatitis (DD), which is likely the most accurate terminology for this disease. Digital refers to the digit or claw and dermatitis simply means inflammation of the skin. Thus, “digital dermatitis” is a very descriptive name.

The Cause or Causes

Despite its known existence for nearly 40 years, the precise organisms responsible for this disease are not entirely known. Early reports of digital dermatitis suggested a viral

etiology because of the wart-like appearance of lesions. However, no one has been able to detect viruses associated with DD. Further evidence of a non-viral cause is the favorable response observed following antibiotic treatment. Lesions, lameness and pain all regress rapidly following treatment with antibiotics. If the cause were a virus this would not occur.

Investigations in the US and Europe consistently identify bacterial spirochetes (spiral-shaped bacteria) in properly stained sections of DD tissue. The question is do these organisms initiate the disease or infect the lesions secondary to other invaders. The problem is further complicated by the fact that there are many types of bacteria present in DD lesions. Sorting them out and determining their significance is an extremely complex process. Finally, assuming we find the precise causes, will we be able to construct a vaccine or more effective treatment strategy? There are many questions to be answered in the process of finding permanent solutions for this disease.

Characteristics of the Lesion

The lesions of DD typically occur on the skin of the plantar aspect of the rear foot adjacent to the interdigital cleft, or at the skin-horn junction of the heel bulbs. On front feet lesions are often found adjacent to the dew claws or bordering the dorsal (front) interdigital cleft. Most lesions are circular or oval with clearly demarcated borders. Hypertrophied hairs often surround the lesion borders and should be distinguished from epithelial outgrowths that look like long hairs extending from the surface of chronic lesions. Chronic lesions without these epithelial outgrowths are generally thickened and have a granular surface.

Figure 1. Severe lesion associated with the planatar interdigital cleft.,

Lesions are very tender and even a mild disturbance of the inflamed tissue tends to result in extreme discomfort and mild to moderate bleeding. Cows will alter their posture and/or gait to avoid direct contact between lesions and the floor or other objects. This avoidance behavior is one of the best visual indicators of a DD lesion. These pain avoidance adaptations also lead to abnormal wear of the weight bearing surface of affected claws. Lesions associated with the plantar interdigital cleft usually cause the cow to shift weight bearing toward the toe. This results in increased wear at the toe, decreased wear in the heel and an overall reduction in the weight bearing surface of the affected claw. When lesions occur on the dorsal (front) aspect of the foot, cows respond by altering posture and weight bearing resulting in overgrowth and extension of the toe and greater wear in the heel. These alterations in shape of the claw generally require correction at the time of trimming.

Atypical (Uncharacteristic) Lesions of Digital Dermatitis

"Invasion of the corium by DD" Exposure of the corium, whether as a consequence of the normal pathogenesis of claw lesions (white line disease and ulcers) or secondary to corrective trimming procedures, is often the prelude to development of a very painful DD lesion. It shares similar characteristics to the more frequently observed skin lesion; that is, a granular and sometimes whitish surface. Trimmers often refer to the condition as "hairy attack", referencing its invasion of the corium rather than the skin above the claw horn capsule. Lesions tend to have well defined borders and depending upon chronicity may be slightly raised in comparison to adjacent unaffected corium tissues. The affected areas are particularly sensitive such that even a mild disturbance results in a very overt pain reaction from the cow. Stained sections of these lesions demonstrate large numbers of bacterial spirochetes typical of DD.

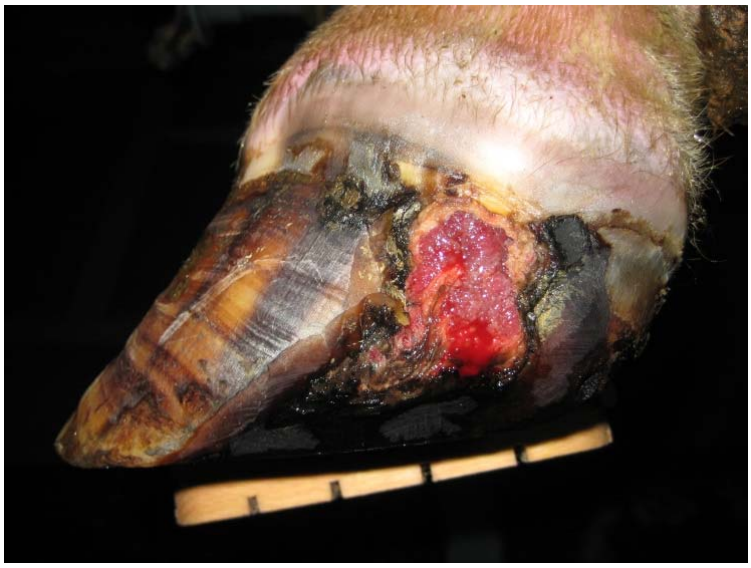

Figure 2. Atypical lesion of digital dermatitis on the corium secondary to corrective trimming of a claw lesion.

The atypical lesion of DD is often observed in association with non-healing claw lesions. Experience with treatment suggests that healing, as evidenced by the formation of new horn over the affected area, is not likely to occur until the infection is under control. Therefore, some form of topical antibiotic therapy is necessary to affect recovery. Researchers from Tennessee and Iowa have also observed that the inflammation associated with this condition may in itself be a significant complication in successful treatment of these lesions. Therefore, they suggest that the inclusion of an anti-inflammatory agent along with the antimicrobial be included in therapy of these lesions.

Figure 3. Typical udder sore on anterior aspect of the mammary gland

"Udder Sores" also known as bovine ulcerative mammary dermatitis, mammary necrotic dermatitis, udder

seborrhea, and udder foul are typically observed in the ventral abdominal wall between the front quarters of the cow's mammary gland. It is described as a moist exudative dermatitis with a characteristic pungent foul odor. Researchers report an increased prevalence of this condition in herds suffering from severe problems with DD. This link has been strengthened by several studies in recent years that have identified bacterial spirochetes similar to those of DD in sections of tissues recovered from these lesions. While further research is necessary to definitively establish DD as the most likely cause of udder sores, previous work demonstrating transmission of DD from foot-to-foot by direct contact suggests that a similar mode of transmission from foot-to-udder is possible. Indeed, some theorize that the pathogens causing DD may be transferred from an infected foot to the abdominal skin as the downside leg of the cow makes contact with the udder and ventral skin when the cow lies down.

Occurrence of the Disease in Herds

Housing, environment and management conditions most consistently identified as underlying causes of DD include: large herd size, wet and muddy corrals, and the purchase of replacement animals. Other factors cited as contributors to DD included: use of a footbath, housing on grooved concrete, use of a trimmer who trimmed feet at other farms, and failure to clean and sanitize equipment between uses on cows. Although the latter study suggested important relationships between the occurrence of DD and various management practices, it did not distinguish between cause and effect. In other words, considering the relationship of footbaths and DD for example, analysis of the data did not establish that DD was caused by footbaths or vice versa. One would have to conclude however, that housing and environmental hygiene are important factors in control of this disease. Furthermore, based on the above studies the importance of hygiene could be extended to those who provide foot care services (veterinarians and/or trimmers) on dairy farms.

Highest incidence rates of DD are usually observed in early lactation. In some herds this is due to extremely high rates of DD in pre-fresh home-grown or off-site raised heifers. Herds that purchase replacements often fail to request DD-free animals or properly inspect purchased animals for the presence of DD lesions before introducing them into their herds. The transition from a non-lactating to a lactating state represents one of the more stressful periods in a cow's life. She must adapt to the physiological changes associated with the initiation of lactation, adjust to changes in housing and feeding conditions, and successfully respond to battles for dominance amongst herd mates. Finally, some have suggested that one of the potential causes of a higher incidence of DD in the early postpartum period may be due to peri-parturient immune suppression.

The possible reservoirs and mode of transmission of DD are largely unknown, but assumed to be clinically and sub-clinically infected cows and fomites. The plantar interdigital cleft between the claw heels provides an excellent environment for the bacterial spirochetes most commonly isolated from lesions, and is therefore likely a significant reservoir. Attempts to reproduce the disease under controlled conditions has

proven difficult, but has been accomplished in young heifers. Experimental transmission was achieved by the placement of skin scrapings from DD lesions under a bandage designed to create an oxygen-depleted and moist micro-environment in the intended anatomical site. Typical lesions were observed after a period of several weeks.

Effects of Digital Dermatitis on Performance

Few studies have attempted to assess the effects of DD on performance. A US study found that milk yield in cows affected with DD was 338 lb (153.3 kg) less than that produced by healthy cows; however the difference was not significant. An earlier study conducted on a 600-cow dairy in Mexico had similar results. Cows affected with DD produced 268 lb (121.6 kg) less milk than their unaffected herd mates; but as in the previous study this difference was not significant. There were, however significant effects on reproductive performance. For cows affected with DD the calving to conception interval was increased from 93 to 113 days and average days open were increased by approximately 14 days as compared with non-infected herd mates.

Treatment and Control Strategies

Past approaches to therapy included: 1) surgical excision, 2) footbaths 3) topical treatment with various disinfectants, and antibiotic solutions, 4) cryosurgery, and electrocautery, 5) topical treatment under a bandage, and 6) systemic antibiotic therapy. With the possible exception of cryosurgery and electrocautery, most of these treatments have a place in the management of this condition. However, they may not be practical in some situations.

Topical spray-on treatment with antibiotic and some non-antibiotic preparations have been shown to be effective when used in a scheme of consistent daily treatment for a period of 8-10 days over a 2-week period. The major disadvantages to topical treatment are that it is labor intensive and lesions occurring in the interdigital space are usually missed by spraying procedures. Footbath solutions are more likely to reach lesions in the interdigital space and offer a great deal more treatment convenience; but footbathing done right also requires some intensive management effort. Footbathing is also quite inefficient and costly. For example, all cows must parade through the

Figure 4. Topical spray application of oxytetracycline for control of digital dermatitis.

footbath in order to get the 10 or 15% of animals that may need treatment. Topical spray on the other hand, allows treatment of only those affected as long as the lesions one is attempting to treat are visible. Response to topical antibiotic treatment (topical spray or in combination with topical antibiotics under a bandage) is also influenced by the anatomic location of lesions. A Florida study demonstrated that lesions occurring on the plantar interdigital cleft were less likely to respond compared with lesions occurring on the heel bulbs or dewclaws. Limited evidence also suggests that response to therapy may be influenced by lesion maturity and possibly antibiotic resistance patterns of etiologic agents. Topical antibiotic treatment under a bandage is particularly effective with most cows showing remarkable improvement within 24-48 hours. However, concerns for the timely removal of bandages have caused some to recommend topical antibiotic treatment with a solution or paste without the use of a bandage. Bandages also increase the possibilities of foot injury from wraps that are applied so tight that they interfere with normal blood circulation in the foot. A Canadian study by Higginson and co-workers compared topical treatment with topical oxytetracycline under a bandage with a topical oxytetracycline paste and no bandage. Results demonstrated a significant advantage to treatment versus no treatment but when results between those treated topically with a bandage or no bandage there was no statistically significant difference between the treatment groups.

Proper application of topical treatments has the potential advantage of reaching lesions affecting the interdigital skin and other areas that may not be accessible to topical spray or even footbath treatment. But, in the end, most agree that it's a combination of these procedures that is most likely to be effective in managing DD lesions.

Topical Treatment - The European Experience

For many years the recommended therapy for DD was surgical removal of the lesion and trimming of the claw as needed. However, the more popular treatment used in Europe during the early 1980's was a combination product (not available in the US) consisting of topical oxytetracycline hydrochloride and gentian violet. The treatment procedure first described consisted of properly restraining the affected animal in a foot-trimming chute whereby the foot was elevated or situated to permit examination. Once the diagnosis of DD was confirmed, the lesion was thoroughly cleaned and then sprayed with the combination product. Efficacy was reported to improve to near 90% if the operator allowed the first topical treatment to dry and follow with a second topical treatment before turning the cow loose. Subsequent study demonstrated that the active ingredient in this combination product was oxytetracycline and that this product alone was sufficient to affect recovery. It is this observation that lends support to the idea of treating these lesions without the use of a wrap or bandage.

Vaccination for Control of Digital Dermatitis

Because of high recurrence rates and the inability to conveniently treat high risk groups of animals (such as growing heifers or feedlot cattle), an effective vaccine for control of DD is highly desirable. Results from early studies of a Treponema bacterin for control

of DD in cattle concluded that immunization could reduce clinical disease. In contrast, German researchers found no benefit from a vaccine containing herd-specific pathogens including *Treponema sp*. Similar results were observed in a recent US field trial which found no therapeutic or prophylactic benefit from vaccination with a Treponema bacterin. At the present time, there are no vaccines commercially available for control of digital dermatitis.

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HEAT DETECTION AND THE USE OF ACTIVITY MONITORS

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Introduction

Since the 1990s timed AI protocols have been developed to improve the AI submission rate (number of cows that receive AI divided by the number of cows that are eligible to be inseminated over a 21 d interval), also known as heat or estrus detection rate, of lactating dairy cows. More recently, a better understanding of reproductive physiology has resulted in timed AI protocols that may result in pregnancy per AI (**P/AI**) of up to 45% in high producing lactating dairy cows (Santos et al., 2010; Souza et al., 2008). Nonetheless, the greatest benefit of timed AI protocols to reproductive performance of dairy herds is increased AI submission rates. Thus, often the decision of whether or not to use timed AI protocols is based on the AI submission rates achieved when AI occurs based only on estrous detection (**ED**). Other factors like accuracy of ED and compliance to the timed AI protocols chosen are also important to the reproductive performance.

Upon the advent of timed AI protocols many suggested that daily ED of lactating dairy cows would no longer be necessary. Timed AI protocols make use of reproductive hormones like GnRH, prostaglandin (**PG**) $F_{2\alpha}$, and progesterone (**P4**). The use of these hormones for reproductive management of dairy cows may undergo scrutiny by consumers similar to what has been observed in regards to the use of antimicrobials possibly limiting their use. The recent growth in number of companies commercializing activity monitors for detection of estrus has resulted in several companies claiming that the implementation of such activity monitors would eliminate the need for timed AI protocols.

The goal of this brief review is to evaluate whether reproductive programs of lactating dairy cows may be solely dependent on timed AI protocols or AI on detected estrus. A few examples of dairies that have attempted to eliminate one or the other will be given, but I caution that some of these examples are merely data extracted from on farm software and not the result of controlled studies.

The Challenges of Estrous Behavior for Lactating Dairy Cows

Unquestionably lactating dairy cows have reduced expression of estrus compared with dairy heifers and beef animals because of physiological characteristics, often because of increased incidence of pathological conditions, and because of management.

Immediately postpartum, cows undergo physiological anovular condition characterized by the lack of ovulation and formation of a corpus luteum (CL) until approximately 25 to 30 days postpartum (Butler, 2000). However, cows that have postparturient diseases and undergo more severe loss of body condition score (BCS) have more prolonged anovular condition. Cows that had no change in BCS from calving to first postpartum AI (approximately 65 DIM) and cows that lost < 1 unit of BCS from calving to first postpartum AI were 2.0 and 2.4 times more likely, respectively, to be cyclic by 65 DIM than cows that lost > 1 unit of BCS during this period (Santos et al., 2009). Furthermore, cows diagnosed with mastitis early postpartum (mastitis = 39 vs healthy = 32 d; Huszenicza et al., 2005) and cows diagnosed as lame within the first 30 DIM (lame =34 vs healthy = 29 d; Garbarino et al., 2006) had prolonged anovular condition than healthy cows. Postponed resumption of ovarian cycles results in delayed establishment of pregnancy because of reduced AI submission rates and reduced P/AI (Chebel et al., 2010; Santos et al., 2009).

Limited access to open lots/dirt lots also seems to be a limiting factor for AI submission rate among lactating dairy cows. Vallies and Britt (1989) demonstrated that mounting activity was 15-fold greater for lactating dairy cows with access to open lots than cows housed solely on concrete.

Onset of lactation affects expression of estrus by reducing concentrations of estradiol. Lopez et al. (2004) demonstrated that cows with greater milk yield (102.1 ± 0.9 lb/d) had reduced duration of estrus (6.2 ± 0.5 vs 10.9 ± 0.7 h) and reduced number of mounts during estrus (6.3 ± 0.4 vs 8.8 ± 0.6 mounts) compared with cows with reduced milk yield (73.7 ± 0.7 lb/d). Furthermore, the same group demonstrated that high producing dairy cows (103 ± 2.2 lb/d) had reduced estradiol concentration on the day of estrus (6.8 ± 0.5 vs 8.6 ± 0.5 pg/ml) despite having larger follicles (18.6 ± 0.3 vs 17.4 ± 0.2 mm) compared with low producing dairy cows (71.1 ± 1.3 lb/d; Lopez et al., 2004). This resulted in reduced length of estrus (7 ± 1.1 vs 11.9 ± 1.4 h) and number of mounts during estrus (6.5 ± 0.9 vs 9.8 ± 1) for high producing cows compared with low producing dairy cows (Lopez et al., 2004). Even though the reasons for the reduced estradiol concentrations of estradiol during estrus in lactating dairy cows are not completely elucidated, the currently most accepted hypothesis is that the elevated dry matter intake of high producing cows, necessary to meet nutritional requirements of lactation, results in greater blood flow through the liver, the most important site of steroidal hormones catabolism. In a series of experiments, Sangsritavong et al. (2002) demonstrated that onset of feed intake resulted in significant increase in blood flow to the liver and that the increase in blood flow was dependent on amount of feed consumed (Figure 1). Furthermore, lactating dairy cows fed 7.8 lb of dry matter had greater clearance rate of P4 at 1 and 2 h after feeding compared with unfed cows (Sangsritavong et al., 2002). Cows fed 23.4 lb of dry matter had greater P4 clearance rate

from 2 to 4 h after feeding than unfed cows, whereas cows fed 33.4 lb of dry matter had greater P4 clearance rate from 1 to 4 h after feeding compared with unfed cows (Sangsritavong et al., 2002). Similarly, lactating dairy cows fed ad libitum had greater estradiol clearance rate from 2 to 4.5 h after onset of feeding compared with unfed cows (Sangsritavong et al., 2002).

Clearly, physiological and pathological conditions share the blame for reduced AI submission rates among lactating dairy cows. Because of the great importance of AI submission rate to the overall reproductive efficiency of lactating dairy cows, in herds where adequate AI submission rates are not achieved, different ED and/or AI submission strategies (i.e. timed AI protocol) should be implemented.

Estrous Detection and Timed AI protocols: Complementary not Mutually Exclusive

In one comprehensive survey conducted in 103 dairy herds from at least 12 states, 74.8% of the herds indicated that an estrus/ovulation synchronization program for first postpartum AI was implemented (Caraviello et al., 2006). When data from 33 million inseminations of Holstein and Jersey cows from Dairy Herd Improvement Association herds were analyzed, however, it was estimated that the percentage of herds that did not use synchronization protocols was 94.8% in 1996 and 72.5% in 2005 (Miller et al., 2007). Thus, it is clear that a lot of variability exists in regards to implementation of timed AI protocols let alone the types of timed AI protocols used.

In general, the implementation of timed AI protocols results in reduced intervals from parturition to first postpartum AI, reduced variability in interval to first postpartum AI, and may reduce the interval from parturition to establishment of a new pregnancy (Miller et al., 2007). These beneficial results, however, are highly dependent on the base line reproductive performance of the herd before adoption of such protocols. Simply putting it, herds that achieve

good AI submission rates and P/AI without timed AI protocols do not necessitate the latter. In the opinion of this author, however, only when P/AI achieved through AI on estrus is extremely poor (poor ED accuracy) should programs based 100% on timed AI protocol be recommended. This is simply a matter of mathematics. Even though 100% AI submission rate may be achieved in the first 21-d cycle after the end of the voluntary waiting period (VWP) when 100% of cows are inseminated at fixed time at first postpartum AI, pregnancy diagnosis is not possible to be conducted until 25 d after AI at the earliest. Thus, re-insemination of nonpregnant cows could only occur as early as 28 d after a previous AI, resulting in the 21-d cycle immediately after AI with AI submission rate of 0% and the following 21-d cycle with AI submission rate of 100%. Thus, herds with 100% timed AI would struggle to achieve AI submission rate greater than 60%, depending on P/AI.

Therefore, the question that must be answered is: what are the breakeven points in the decision for 100% timed AI, 100% ED, or both? To answer that question, we must take into consideration published research and the outcomes obtained with different timed AI protocols and the reported P/AI following AI on estrus. It is important to remind the readers that the numbers presented in peer-reviewed manuscript are often inflated because they result from well controlled studies and often sick cows (i.e. extremely lame, low BCS, etc.) and cows that fail to receive the appropriate treatments are removed from the study.

One of the first experiments to evaluate the economic benefits of reproductive strategy based on ED or timed AI was conducted in Germany (Tenhagen et al., 2004). In this experiment, cows from two herds were either only inseminated based on estrus or were inseminated at fixed time until approximately 200 DIM. In the herd in which AI submission rate of cows inseminated on estrus was 29%, the timed AI protocol resulted in significant improvements in AI submission

rate (65%) and pregnancy rate (14 vs 25%). On the other hand, in the herd in which AI submission rate of cows inseminated on estrus was 55%, the timed AI protocol slightly increased the AI submission rate (70%), but had no significant effect on pregnancy rate (25 vs 29%). Consequently, in the herd with poor AI submission rate of cows in the ED treatment the addition of timed AI to the reproductive management resulted in reduced cost per pregnancy generated (€ 363 vs € 264). On the other hand, the cost per pregnancy generated was similar among cows submitted to the ED protocol (€ 251) or the timed AI protocol (€ 272) in the herd in which AI submission rate of cows in the ED treatment was 55%. This was one of the first experiments to suggest that in herds that only inseminate cows in estrus and have AI submission rate greater than 55% the use of timed AI protocols may not be necessary.

In two recent manuscripts, researchers compared the economic outcomes of reproductive strategies based on ED, timed AI, or a hybrid between ED and timed AI. These experiments used modeling techniques to simulate the economic return of the different reproductive programs. Giordano et al. (2001) evaluated economic return of reproductive programs for lactating dairy cows based on ED, the double Ovsynch protocol for first AI and the Ovsynch protocol for resynchronization of cows starting 32 d after the previous AI (**DO-Res**), and the double Ovsynch program for first AI and resynchronization (**DO-DO**). The DO-Res ($ 17 cow/year over the cost of the ED program) and the DO-DO ($ 21 cow/year over the cost of the ED program) programs were more expensive than the ED protocol. On the other hand, the DO-Res and the DO-DO protocols resulted in income per cow/year $ 45 and $ 69 greater, respectively, than the ED protocol. The authors, however, based their calculations of economic return on P/AI results from one study and on farm data for ED cows. As such, P/AI to first AI and resynchronization were 45 and 30%, respectively, for DO-Res protocol, 45 and 39%, respectively, for DO-DO protocol, and

33 and 30%, respectively, for the ED protocol. It is not surprising, therefore, that with such differences in fertility, the DO-DO and the DO-Res protocols resulted in greater economic return than the ED protocol. Nonetheless, P/AI of cows subjected to timed AI is not significantly greater that P/AI of cows inseminated following synchronized estrus based on several published manuscripts that did not use the Double-Ovsynch protocol (Chebel and Santos, 2010; Santos et al., 2009; Santos et al., 2004a; Tenhagen et al., 2004).

Galvão et al. (2012) modeled reproductive performance and economics based on the adoption of one of ten breeding programs. The breeding programs evaluated were based on ED or timed AI and taking into consideration differences in ED efficiency (40 or 60%) and accuracy (85 or 95%), compliance to injections of the synchronization protocols (85 or 95%), and milk price ($ 0.33 or $ 0.44/kg). The reproductive programs evaluated were ED with differing ED efficiency and accuracy, timed AI for all with differing compliance to injections, and timed AI for first AI with differing compliance followed by ED with differing ED efficiency and accuracy. Pregnancy per AI for first AI was assumed to be 33.9% and P/AI of subsequent AI decreasing by 2.6% for every AI, pregnancy loss was assumed to be 11.3%, cows were deemed not eligible for insemination if nonpregnant after 366 DIM, and were culled by 450 DIM if not pregnant. All costs associated with the reproductive programs and feeding were taken into consideration. Milk price was set at $0.33 or $0.44/kg, cull cows were sold for $0.65/kg of live weight, and calves were sold for $140/calf. Under these assumptions, when the herd used timed AI for first postpartum AI with 95% compliance to injections and ED for subsequent AI with ED with 60% efficiency and 95% accuracy the greatest 21-d cycle pregnancy rate was achieved (Figure 2A; Galvão et al., 2012). Similarly, this reproductive program resulted in the shortest median days to

pregnancy (113; Figure 2B) and the greatest profit per cow/year (profit of $375/cow for milk price = $0.33/kg; profit of $1,616/cow for milk price = $0.44/kg of milk).

Therefore, postponing re-insemination of cows that return to estrus in order to submit them to timed AI protocols seems illogical because of the consequent increased interval to re-insemination. As mentioned before, the only reason to avoid insemination and, particularly, re-insemination in estrus is poor ED accuracy, which results in reduced P/AI of cows inseminated in estrus. Dairy farms in the USA commonly utilize timed AI protocols in association with insemination on estrus. Approximately 55% of dairy farms rely primarily on detection of estrus as the major method to inseminate cows (NAHMS, 2009). Among the winners of the award for Excellence in Reproductive Management of the Dairy Cattle Reproductive Council between the years of 2009 to 2011, 21 out of 24 used AI on detected estrus associated with timed AI protocol, 2 out of 24 used only timed AI protocols, and 1 out of 24 used only AI on detected estrus.

Recently, several companies have started to commercialize in the USA activity monitors for detection of estrus. These activity monitors are placed in the collars or legs of cows and determine the walking distance and pattern of cows. Once a cow presents an excessively elevated walking patter, the system flags the cow as a suspect for estrus. These systems have been used in other countries (i.e. Israel) for several years and have presented very good results. Interestingly, however, it has been proposed that implementation of electronic methods for detection of estrus would eliminate the need of any timed AI protocol because of its efficiency and accuracy. This is a somewhat ambitious claim, particularly considering the physiological and pathological challenges that affect onset of estrus and estrous behavior of high-producing lactating dairy cows. Anovulation, low estrous expression associated with high-production, and other less prevalent abnormalities such as persistent corpora lutea or pregnancy loss after day 21 of the

preceding AI would all reduce the efficacy of estrous detection and result in more nonpregnant cows being diagnosed at the day of pregnancy diagnosis. Some have claimed that electronic monitoring systems can detect 99% of the cows that display estrus. This high sensitivity should not imply that electronic monitoring systems will result in 99% AI submission rate. The key issues here are the cows that remain anovular after the end of the VWP (10 to 50% of cows depending on interval from calving, herd, parity, etc.) and cows that are not pregnant from previous inseminations that will not return estrus within 21 d after a previous AI. Therefore, anovular cows and the cows with abnormal inter-estrus interval would not be detected in estrus and AI submission rates, which are calculated using 21-d cycles, would likely be approximately 50 to 60%, and not any higher.

Nonetheless, until recently there were no controlled experiments to determine whether activity monitors could eliminate the need for time AI protocols completely. Two recent experiments, however, indicated that activity monitors are not able achieve AI submission rates of 90 to 95% as some companies were claiming for the simple fact that some cows will not display estrus. Valenza et al. (2011) fitted 42 cows with an activity monitor system (collar) and a mounting detection system (Kamar). The cows were synchronized and allowed to come in estrus. Cows were then examined by ultrasound to determine ovarian activity and occurrence of ovulation. In this small experiment, according to activity monitor and mounting detector 67 and 62%, respectively, of cows were observed in heat and ovulated; 7 and 12%, respectively, of cows were not observed in heat and ovulated; 5% of cows were observed in heat and did not ovulate; and, 21% of cows were not observed in heat and did not ovulate. Therefore, based on an activity monitor system and a mounting detection system 28 to 33%, respectively, of cows were not observed in estrus. Furthermore, considering ovulation as the ‘gold standard’, cows that ovulated

and were in estrus were +/+, cows that did not ovulate and were in estrus were -/+, cows that ovulated and were not in estrus were +/-, and cows that did not ovulate and were not in estrus were -/-. Thus, the activity monitor system and the heat detection system resulted in sensitivity of 91 and 84%, respectively, specificity of 81%, positive predictive value of 93%, and negative predictive value of 75 and 64%, respectively. Therefore, based on this small experiment the activity monitor and mounting detection system had similar performance.

In a study presented at the 2012 American Dairy Science Association, researchers evaluated the insemination pattern and P/AI of cows that were fitted with activity monitors and were submitted to the Ovsynch protocol with ED (Ovs), to the Presynch/Ovsynch with ED (PresOvs), and to the Presynch/Ovsynch protocol without ED (100%TAI; Fricke et al., 2012). In this study, 70% of cows that received the two $PGF_{2\alpha}$ presynchronizing injections were observed in estrus, whereas approximately 57% of cows that were not presynchronized with $PGF_{2\alpha}$ were observed in estrus. The P/AI of cows inseminated in estrus was 30% and the P/AI of cows inseminated at fixed time was 36%. These numbers are very similar to those reported by Stevenson and Phatak (2005), Chebel et al. (2006), Lima et al. (2009), and Chebel et al. (2010). In these studies the percentage of cows that were inseminated in estrus after two presynchronizing injections of $PGF_{2\alpha}$ ranged from 50 to 62%. On the other hand, P/AI of cows inseminated in estrus ranged from 27 to 44% and P/AI of cows inseminated at fixed time ranged from 21 to 41%. The results from these studies suggest that activity monitors may perform just as well as detection of estrus based on tail paint removal and that P/AI of cows inseminated in estrus based on activity or tail paint removal may be similar, these being extremely dependent on farm and personnel.

Field observations of two herds that adopted the activity monitor systems for estrus detection and abolished the use of fixed time AI for first postpartum AI demonstrate that there is a significant risk of increasing significantly the variability in interval to first AI, increasing interval to first postpartum AI, and reducing AI submission rate and pregnancy rate. In figure 3A and 3B, the patterns of first postpartum AI of herds that started using timed AI protocols for first postpartum AI are depicted. In figure 3C and 3D, the patterns of first postpartum AI of herds that stopped using timed AI protocols once they implemented activity monitoring systems are depicted. Although this is not data from controlled studies, it is possible to observe that once timed AI protocols stopped being used in the herds that adopted the activity monitoring system their pattern of first postpartum AI started to resemble the pattern of first postpartum AI before timed AI protocols were widely adopted.

Conclusions

It is widely known that estrous expression and estrous detection of lactating dairy cows are compromised by several physiological, pathological, and managerial factors. The advent of timed AI protocols has resulted in significant improvements AI submission rates, a very important component of reproductive efficiency and perhaps the easiest parameter manipulate with different managerial strategies. Activity monitoring systems are also an exciting tool for the reproductive management of dairy cows that has significant value. Nonetheless, the selection of reproductive strategies should be made in light of estrous detection efficiency and accuracy and in light of availability of facilities and personnel.

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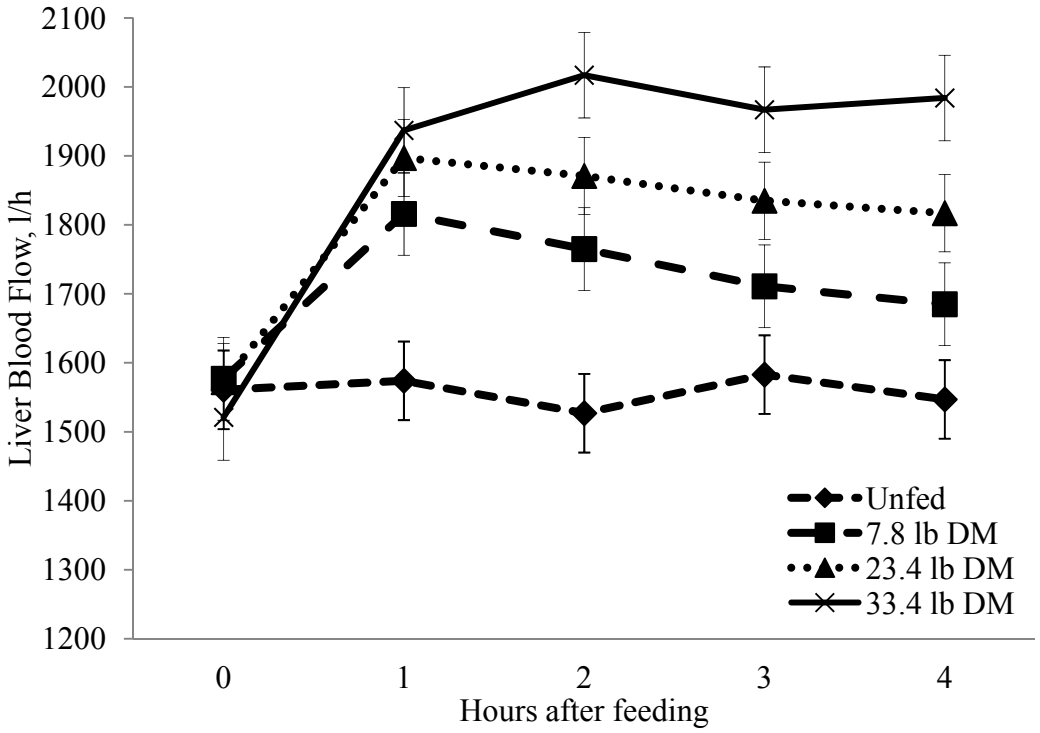
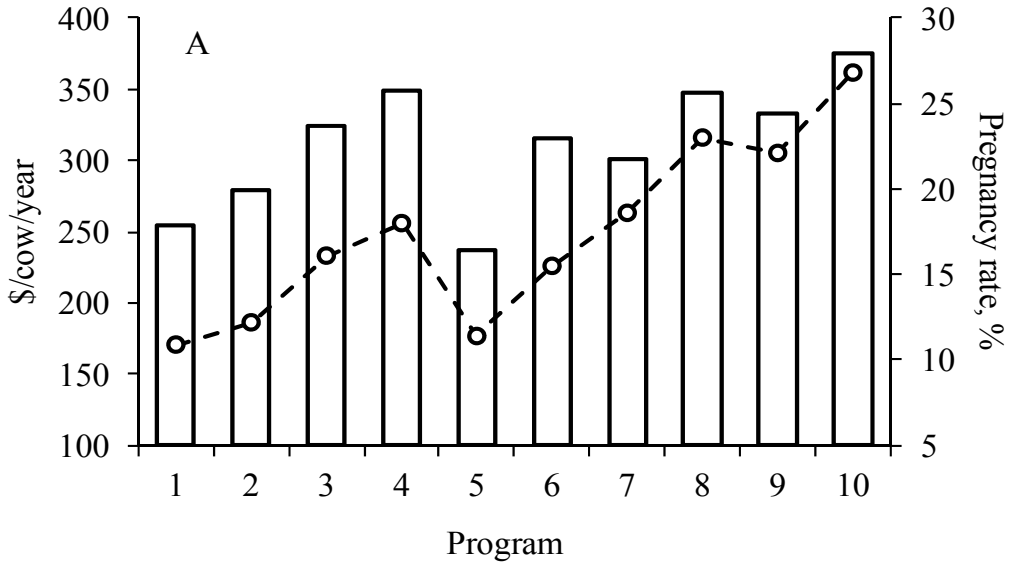


Figure 1. Effect of feed intake on liver blood flow. Adapted from Sangsritavong et al. (2002).


A
400
350
300
250
200
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$/cow/year
30
25
20
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10
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Pregnancy rate, %
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5
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10
Program

Figure 2A.

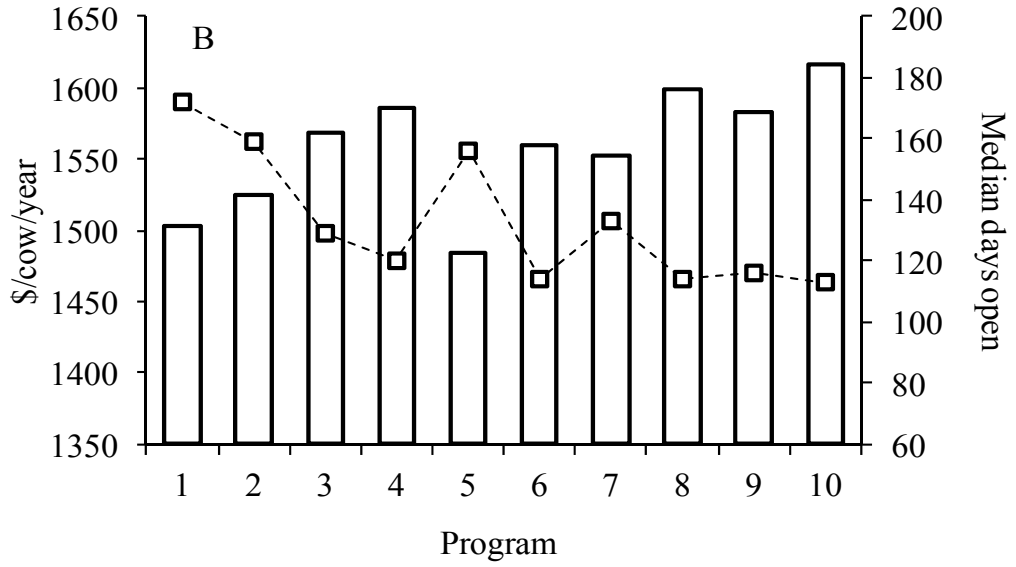

Figure 2B.

Figure 2. Profits per cow per year ($/cow/year) of cows subjected to 1 of 10 breeding programs: 1) ED at 40% efficiency and 85% accuracy; 2) ED at 40% efficiency and 95% accuracy; 3) ED at 60% efficiency and 85% accuracy; 4) ED at 60% efficiency and 95% accuracy; 5) timed AI for all AI (85% compliance); 6) timed AI for all AI (95% compliance); 7) timed AI for first AI (85% compliance) followed by ED at 40% efficiency and 85% accuracy; 8) timed AI for first AI (95% compliance) followed by ED at 40% efficiency and 85% accuracy; 9) timed AI for first AI (85% compliance) followed by ED at 60% efficiency with 85% accuracy; and 10) timed AI for first AI (95% compliance) followed by ED at 60% efficiency with 95% accuracy. In panel A, bars represent the profit per cow per year calculated using milk price at $ 0.33/kg and dashed lines represent the 21-day cycle pregnancy rate. In Panel B, bars represent the profit per cow per year using milk price at $ 0.44/kg (panel B). Dashed lines represent either the 21-day cycle pregnancy rate (panel A) or median days open (panel B). Courtesy of Ribeiro et al. (2012): Adapted from Galvão et al. (2012).

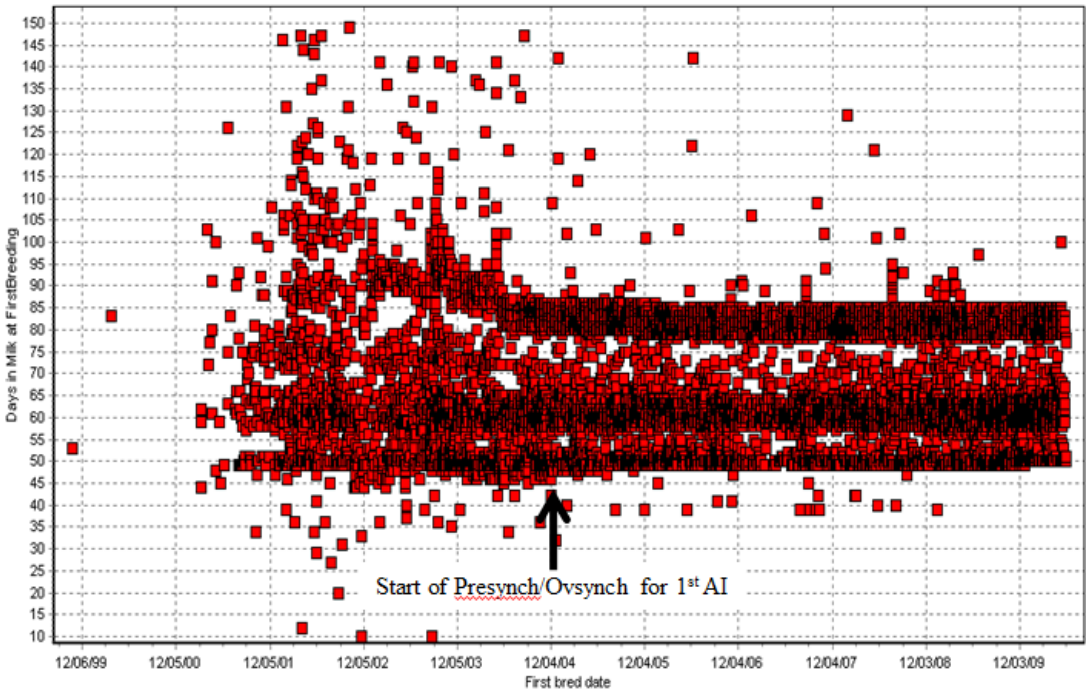

Figure 4A. Pattern of first postpartum AI of a dairy herd in CA (1,600 lactating cows) that implemented timed AI starting December of 2003.

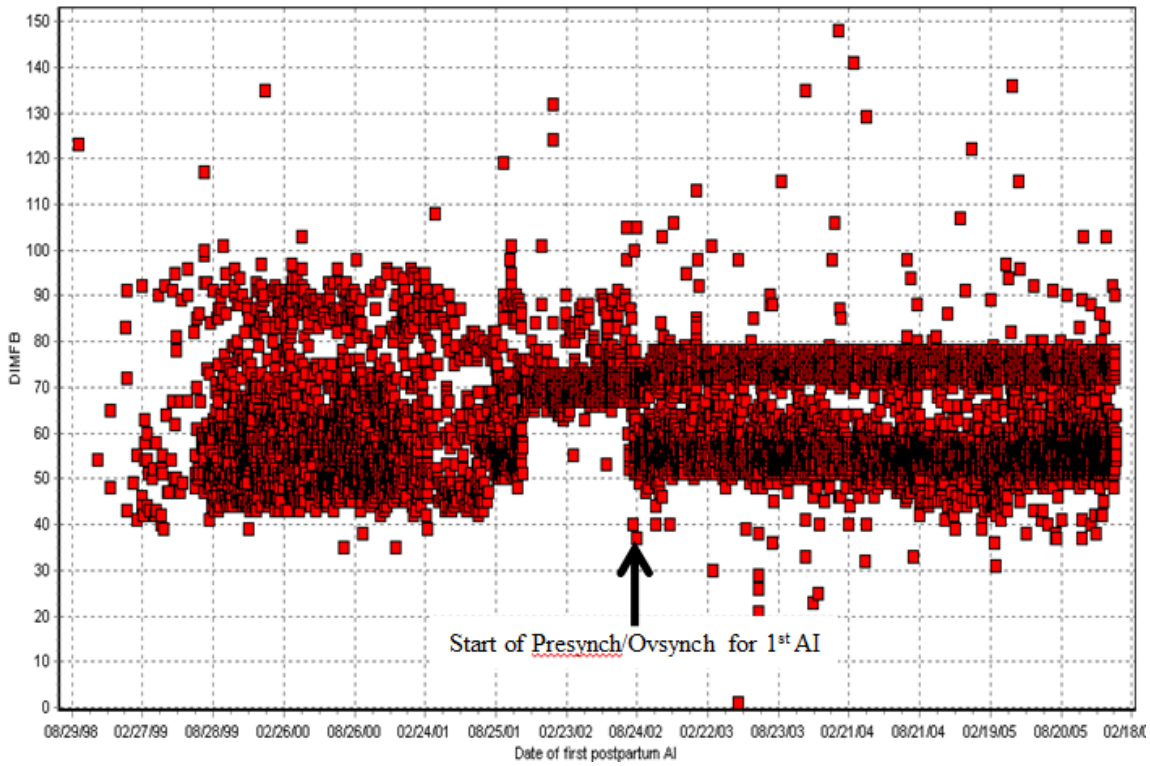

Figure 4B. Pattern of first postpartum AI of a dairy herd in CA (2,300 lactating cows) that implemented timed AI starting August of 2002.

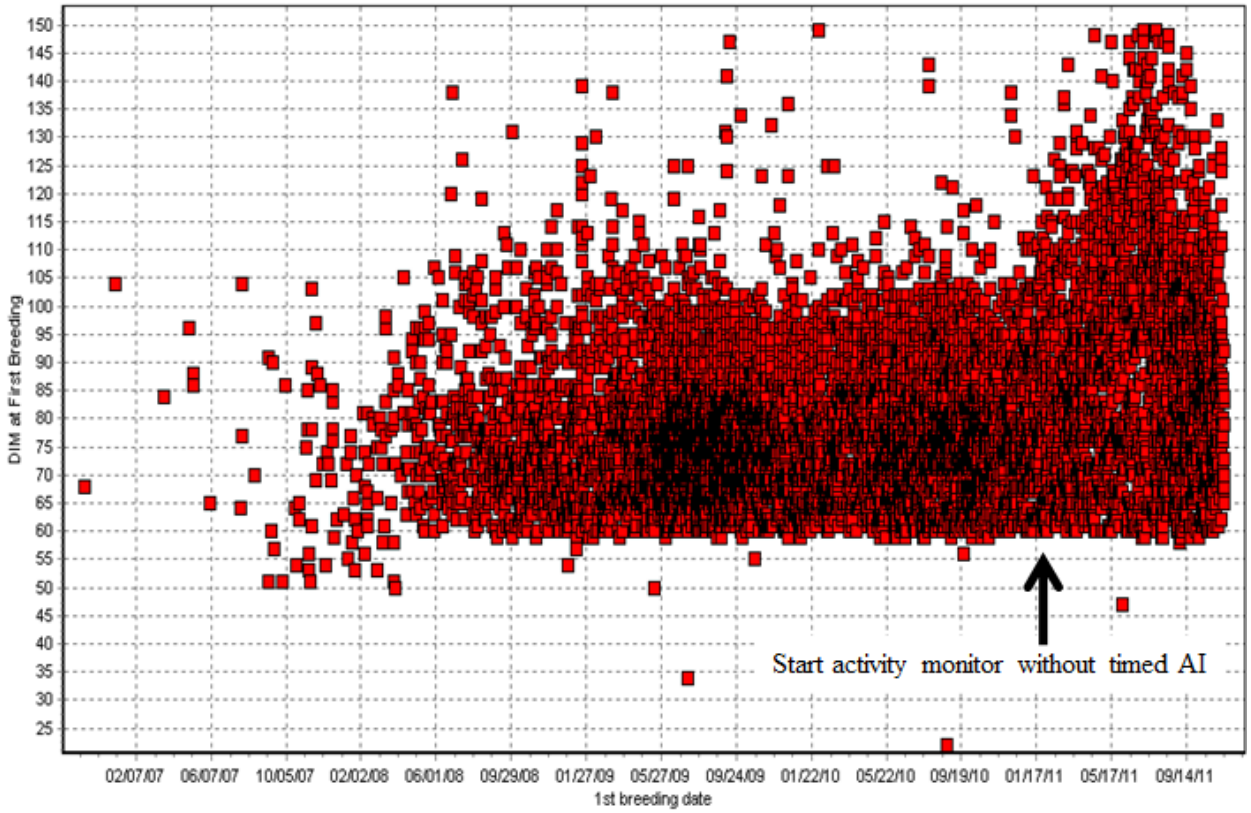

Figure 4C. Pattern of first postpartum AI of a dairy herd in MN (3,100 lactating cows) that implemented the activity monitoring system without timed AI starting January of 2011.

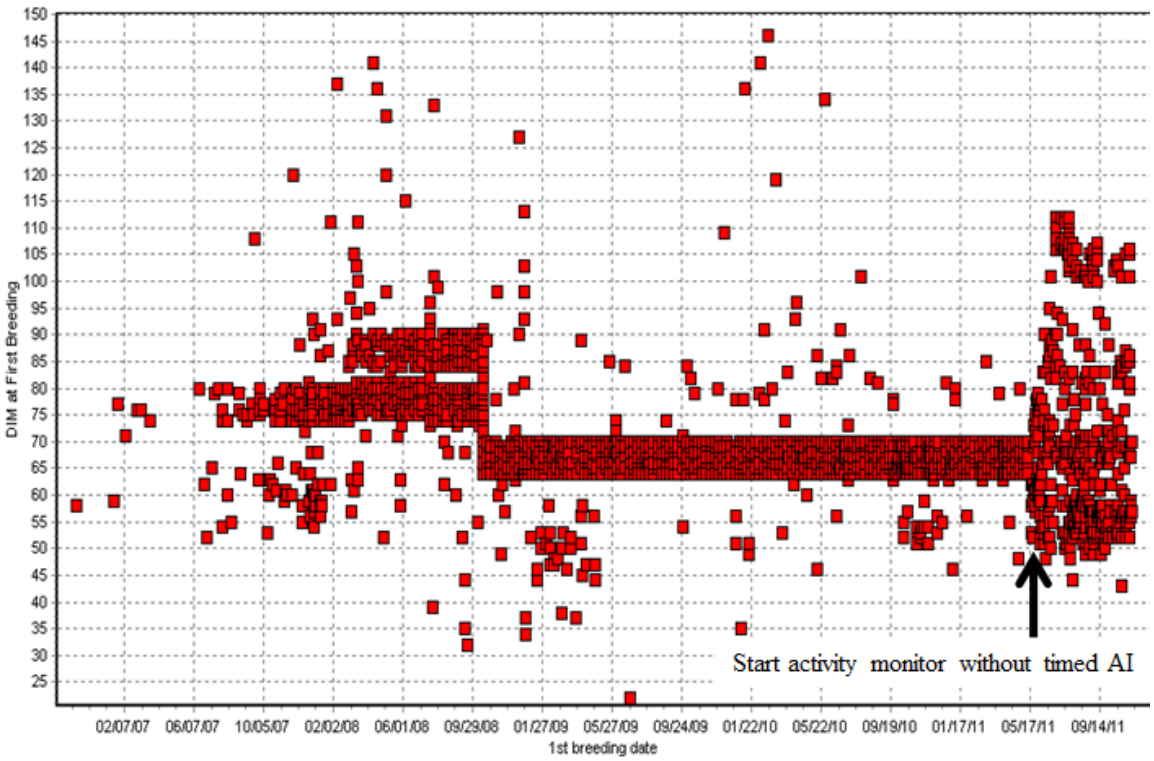

Figure 4D. Pattern of first postpartum AI of a dairy herd in MN (800 lactating cows) that implemented the activity monitoring system without timed AI starting May of 2011.

New developments in pregnancy diagnosis

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Introduction

Pregnancy testing in dairy cattle has evolved over time. The simplest and most definitive test for pregnancy is to wait until the cow gives birth to the calf. This approach is perhaps acceptable for extensive systems but for intensive systems waiting until calving to identify the pregnant or nonpregnant (open) cows takes too long. The desire for an earlier pregnancy diagnosis led to the routine use of rectal palpation of the uterine contents for the purpose of detecting the pregnancy. Although traditionally practiced from 40 to 60 days after insemination or later, pregnancy diagnosis by rectal palpation can be pushed to its limit of detection (30 to 35 days after insemination) to identify open cows sooner. Additional sensitivity can be achieved by using transrectal ultrasound for pregnancy detection. Transrectal ultrasound can be used as early as 25 days after insemination but is more typically applied after day 30 (Fricke, 2002). If performed later (60 to 80 days) then the sex of the calf can be determined when ultrasound is used. Although ultrasound represents a definitive test for pregnancy and can be used to determine the sex of the calf, it requires specialized equipment. The examination generally requires more time than rectal palpation. Regardless of whether rectal palpation or ultrasound is used, an individual with highly specialized training performs the diagnosis. This individual is typically a veterinarian or, in some cases, may be a reproductive specialist that is an employee of the farm.

A changing cattle industry may affect how pregnancy diagnoses are performed in the future. Intensification of reproductive management in dairy herds and the implementation of resynchronization programs are creating the need for more accurate and timely diagnoses of pregnancy. At the same time, there is a shortage of large animal veterinarians in some regions (Jensen et al., 2009). The shortage of large animal veterinarians has put pressure on a limited number of experienced veterinarians to complete a large number of pregnancy diagnoses. Collectively, these factors are creating an opportunity for the application of chemical pregnancy testing (for example, blood and milk-based tests for pregnancy). The cattle industry is clearly moving toward alternative methods of pregnancy diagnosis that do not require skilled practitioners or specialized equipment.

Physiological and theoretical aspects of four tests

If cattle were people then the solution would be simple. The human pregnancy produces a large amount of a hormone called hCG (human chorionic gonadotropin) that passes into the urine and can be detected by a simple lateral flow ELISA test. This test is done by women in their homes. Unfortunately cows do not make bovine chorionic gonadotropin (or any such molecule that is readily detectable in the urine) so a simple test that is similar to the human test is not available. There are, however, a series of candidate molecules associated with pregnancy in cattle (Figure 1). These molecules include: “early pregnancy factor”, interferon-stimulated genes (ISGs), progesterone, and pregnancy-associated glycoproteins (PAGs).

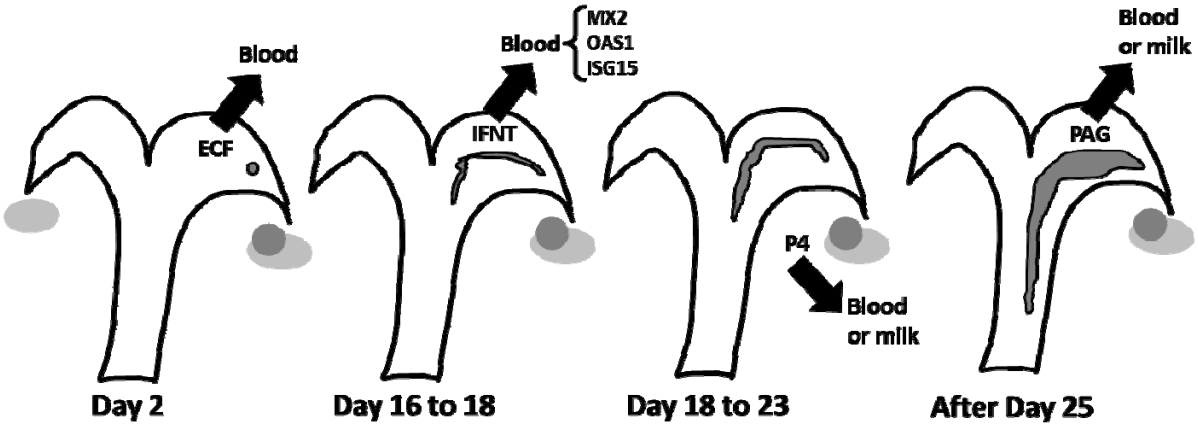

Figure 1. Four chemical tests for pregnancy. Pregnancy can be detected at different intervals after insemination by measuring different chemicals in the blood or milk. In this figure, the uterus, embryo (grey structure within the uterine horn), and ovary (ovoid structure with circular corpus luteum) are depicted. The ECF test (left-most depiction) was reported accurate on day 2 but the test was later shown to be inaccurate at any time. Other tests measure the biological response to interferon-τ (day 16 to 18), progesterone in blood or milk (day 18 to 23), or pregnancy associated glycoproteins in blood or milk (PAG; after day 25). See text for details on the individual tests.

Early Pregnancy Factor

A chemical test for pregnancy termed Early Pregnancy Factor (EPF) or Early Conception Factor (ECF) was proposed and marketed in the 1990's (Concepto Diagnostics, Knoxville,TN). This molecule was supposedly present in the blood of pregnant cattle within two days after conception. The exact nature of this molecule (an immunosuppressive glycoprotein protein) and how it got into circulation were not well defined but nonetheless it could be assayed by using a rosette inhibition test (Nancarrow et al., 1981). A kit for pregnancy diagnosis reached the market but studies found that the kit was unreliable for pregnancy diagnosis (Cordoba et al., 2001; Gandy et al., 2001).

The possibility of performing an early pregnancy diagnosis (within one day after insemination) is, of course, intriguing in as much as it may be possible to resynchronize open cows within one week after insemination. To our knowledge, very little is known about the secretions of the early bovine embryo (within one week after conception). Detection of these secretions in blood, milk, or urine for the purpose of pregnancy diagnosis is an interesting area for investigation. There would theoretically be a large amount of embryonic loss after the diagnosis but the truly nonpregnant cow could be dealt with in a timely manner (within one week after insemination).

Interferon-stimulated gene expression

The early embryo forms a blastocyst and hatches out of the zona pellucida at approximately one week after fertilization. During the second week, it continues to grow, becomes spherical, and during the third week elongates to form the filamentous embryo. It is during this transition from the spherical to elongated form that the embryo produces interferon-τ (INFT). The INFT is produced in large amounts by the embryo after day 14 to signal the mother and establish the pregnancy (Roberts, 2007). The INFT secretion is transitory. It reaches a maximum by 20 to 24 days and is completely gone by day 30 of pregnancy. The IFNT is unlike hCG because its expression is transitory and it does not accumulate in the blood or urine. Thus, IFNT cannot be used for a pregnancy test in the blood or urine of the cow.

Although the INFT cannot be assayed directly in blood, its presence can be detected through its action on leukocytes (white blood cells). Interferon-τ is a cytokine, a class of molecules that has the capacity to stimulate leukocyte function. The leukocyte response to INFT can be monitored by measuring the expression of secondary proteins that are called "interferon-stimulated genes" (ISGs) within leukocytes (Gifford et al., 2007). Examples of ISGs are MX2, ISG15 and OAS1. An example of the ISG response is shown in Figure 2 (data for MX2). In this experiment, dairy cows were blood sampled on days 14, 16, 18, and 20 after insemination and were later diagnosed as either pregnant or open by ultrasound. The RNA from the leukocytes was extracted from the blood and analysed by using a process called "reverse-transcriptase PCR" (RTPCR). The graph shows that the expression of MX2 increases in the leukocytes of pregnant cows particularly on days 18 and 20. This increase in the MX2 represents the leukocytes responding to the IFNT produced by the embryo.

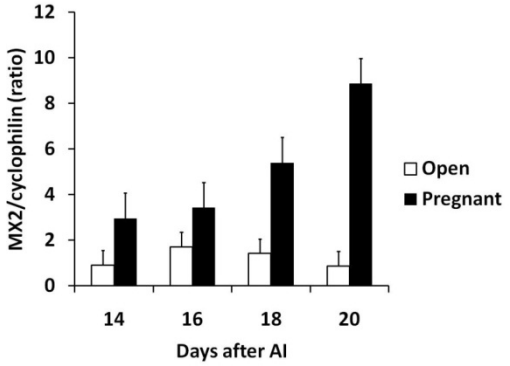

Figure 2. Ratio of MX2 to cyclophilin gene expression in leukocytes isolated from cows on days 14, 16, 18, and 20 after insemination. Compared with open (nonpregnant) cows, the pregnant cows have an increase in the ratio. The increase in MX2 relative to cyclophilin (control gene) can be used as an indication or pregnancy.

Despite their promise as a diagnostic tool for pregnancy detection, the ISGs have not seen commercial application. The current and most reliable assay method involves RNA extraction and RTPCR. The RNA in leukocytes is a highly labile molecule and blood samples would theoretically require special handling before analysis. The RTPCR is a cumbersome and time-consuming laboratory process (relative to ELISA, for example). Although individual cows may give a strong signal by day 18, our experience is that a consistent signal is not achieved until day 20 after insemination. This is only five days earlier than the simpler and perhaps more reliable PAG test (see below). A breakthrough in this area may come if the protein instead of the RNA for ISGs could be measured in blood or milk or if IFNT itself could be measured in blood or milk.

Progesterone monitoring

Measuring progesterone in blood or milk as a method to identify open (nonpregnant) cows was perhaps the first true example of chemical pregnancy testing. If a cow is not pregnant then she will theoretically have a decrease in progesterone at approximately 21 days after insemination. If she is pregnant then her progesterone concentrations will remain elevated. There is excellent physiological underpinning for the progesterone test because cows cannot be pregnant if they have low (less than 1 ng/mL) progesterone 21 days after insemination. The test can be done on the farm (milk progesterone test for dairy cows) or in the laboratory. When done on the farm and with a single sample (for example on day 21) progesterone testing is an excellent method for identifying a truly open cow. If a cows tests low for progesterone then she is not pregnant (progesterone testing has a high negative predictive value). If a cow tests high for progesterone then she may be pregnant (progesterone has a low positive predictive value). The low positive predictive value arises from a number of reproductive cycle-related issues that cannot easily be resolved if a single measurement for progesterone is made.

DeLaval recently introduced a new system (Herd Navigator^{TM}; http://www.delaval.com/en/-/Product-Information1/Management/Systems/herdnavigator/Heat-detection/) that automatically samples cows for milk progesterone. This new system is not available in the United States but is currently in use within Europe and Canada. Milk from cows is sampled automatically based on the reproductive status of the cow. When multiple days are sampled and displayed graphically, estrous cycles are detected (Figure 3; the successive increases and decreases in progesterone are estrous cycles that occur during the postpartum period). It is possible to link the progesterone measurements to activity monitoring so that periods of estrus can be accurately identified. With this system, progesterone can be used to diagnose pregnancy. If a cow is inseminated and her milk progesterone concentrations remain elevated beyond 21 days then she is in all likelihood pregnant. There is limited published data on this new system for its use in pregnancy diagnosis but milk progesterone monitoring within the Herd Navigator system should theoretically be accurate. False positives would occur in cows with persistent corpora lutea; a phenomenon that is relatively rare in cows that are cycling normally.

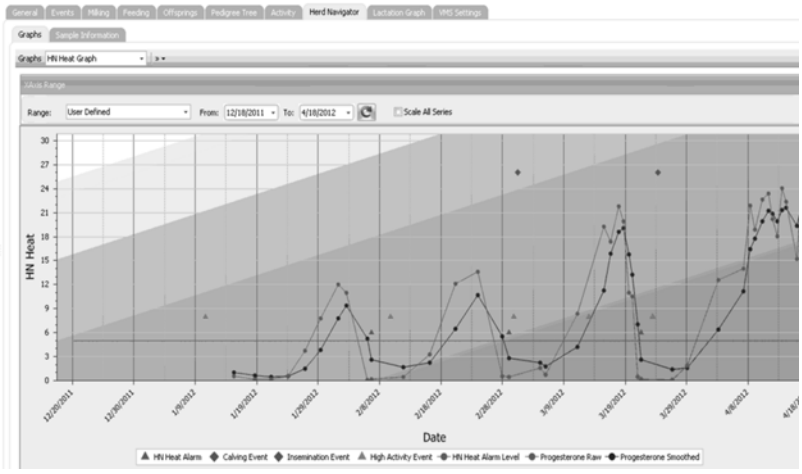

Figure 3. Screen shot of progesterone plot from a postpartum dairy cow (DeLaval Herd Navigator System). A series of estrous cycles are displayed. The cow has an increase in activity (triangles) when progesterone concentrations decrease. A sustained increase in progesterone (final cycle) indicates that the cow is pregnant after AI.

Pregnancy-associated glycoproteins (PAGs)

Binucleate cells are specialized cells of the placenta that migrate and fuse with the epithelium that lines the uterus. Placental proteins synthesized by binucleate cells are secreted into the blood and circulate throughout the cow. One type of placental protein secreted by the binucleate cells is the pregnancy-associated glycoprotein (PAG). The PAGs consist of a large family of more than 20 closely related proteins that are only produced by the placenta (Telugu et al., 2009). They can be detected in the blood of pregnant cows beginning at approximately 25 days after insemination (Green et al., 2005). This family of proteins is expanding in the bovine genome but their function is unknown. Monitoring the concentrations of PAGs in blood or milk is an effective method of pregnancy detection.

The original work on PAGs was done by Sasser et al. (1989) in which they described proteins in the blood of pregnant cows. The protein that they isolated was called "pregnancy-specific protein B" or PSPB. The PSPB is a member of the PAG family of proteins produced by the placenta. The original PAG (PSBP) test is commercially available through BioTracking, LLC (Moscow, ID) or through one of the commercial labs affiliated with BioTracking. The test is trade-named "BioPRYN". Blood samples are collected from cattle that are approximately 30 days after insemination and shipped at room temperature. Data (pregnancy status of individual animals) are returned to the producer via telephone, mail, fax, or email. The test has an extremely high negative predictive value (99.9%; data provided on company website). The high negative predictive value means that if a cow is diagnosed open than she is definitely open. The positive predictive value is also extremely high (approximately 95%) but slightly lower than the negative predictive value. The slightly lower positive predictive value is caused by a small percentage of pregnant cattle that undergo embryonic loss after testing. Pregnant cattle that undergo embryonic loss will initially test positive (pregnant) but will later be found open because the embryo died (Figure 4). The PAGs from the previous pregnancy are found within the blood stream for several months after calving. There is a 2 to 3 month waiting period, therefore, before testing a cow for pregnancy (Figure 4). Virgin heifers can be tested at any time because a positive result cannot be confused with a previous pregnancy.

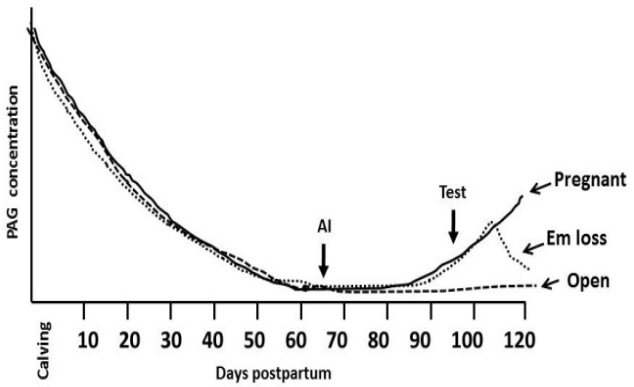

Figure 4. Conceptual diagram of the blood PAG concentrations in postpartum cattle. At calving, the blood PAG concentrations are extremely high. For boPAG-1 (PSPB), 60 to 90 d are required for the PAG from the previous pregnancy to entirely clear the blood stream. In this example, the AI is 65 days postpartum and cows are tested at 95 days. Pregnant cows have elevated PAG whereas open cows do not. A cow that undergoes embryonic loss (Em loss) will have an increase in PAG until the embryo dies. The PAG will decrease in blood after the embryo dies and will require 1 to 2 weeks to clear from the blood stream.

In addition to the BioPRYN test there are two additional commercially available PAG tests. Conception Animal Reproduction Technologies (Beaumont, Quebec, Canada) has partnered with AgSource Cooperative Services and Genex Cooperative (Cooperative Resources International, CRI, Shawano, WI) to market a test called DG29. The second commercially available test is being marketed by IDEXX laboratories (Westbrook, ME). The results for the IDEXX test and BioPRYN test are essentially the same (Figure 5; blood samples collected 25 days after AI). The IDEXX test takes less time to complete (about 3 h) when compared with the traditional BioPRYN test (overnight). Both product inserts state that cows can be tested 28 days after AI. There are differences in terms of when the test can be used. The cows should be at least 60 days postpartum for the IDEXX test and at least 73 days postpartum for the BioPRYN test. We mistakenly tested cows too early with BioPRYN and had two "false positive" diagnoses that were not seen with IDEXX (wells A6 and H1; Figure 5).

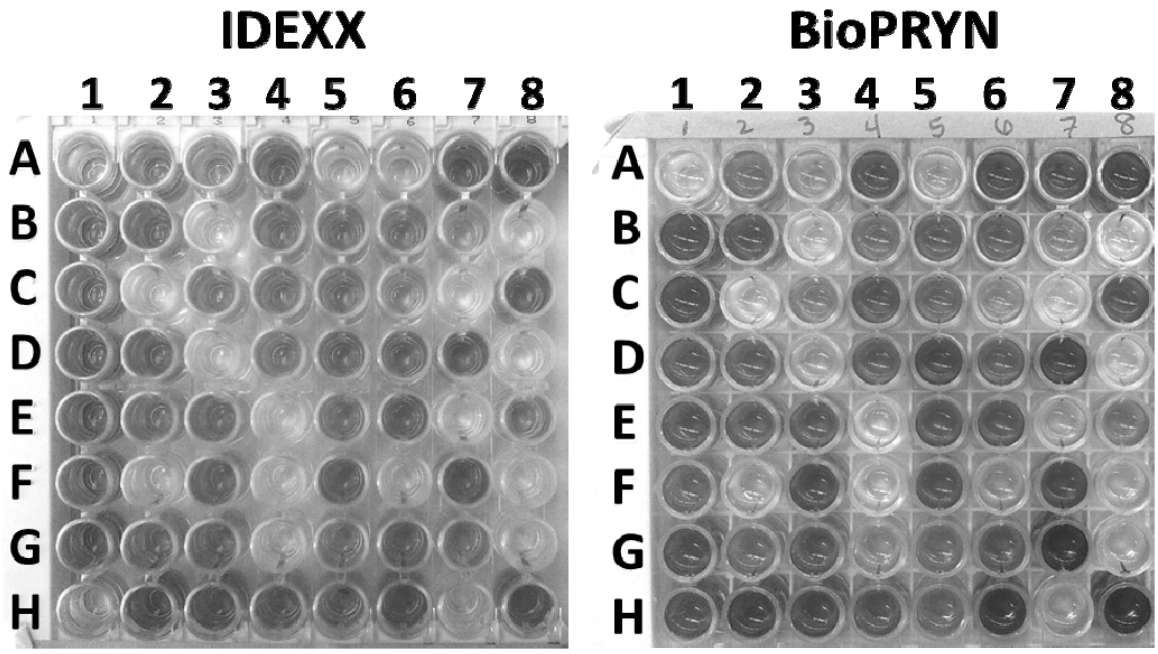

Figure 5. Side by side comparison of IDEXX and BioPRYN pregnancy tests for blood samples collected 25 days after AI. Dark color indicates pregnancy. The tests perform nearly identically. The only difference was for two cows (A6 and H1) where the IDEXX diagnosis was negative and the BioPRYN diagnosis was positive. These two cows were 61 and 70 days postpartum (below the established cut-off for BioPRYN but after the established cut-off for IDEXX).

IDEXX has recently release a milk PAG test. The product insert for the milk test states that it can be used 35 days after AI. Like the blood test, cows should be at least 60 days postpartum. In a recent study, the milk test had a positive predictive value of 99.8% when used in cows that were at least 60 days pregnant (LeBlanc, 2013). The recommendation from the study was that the test can be used to confirm pregnancy in cows that were previously diagnosed pregnant. Any cow with a negative test should be checked by using traditional palpation or ultrasound before additional action is taken. We have tested the IDEXX milk test in our lab (Figure 6). Our

preliminary data indicate that the milk test is equivalent to the blood test in terms of the intensity of the signal and the days after AI that cows can be tested.

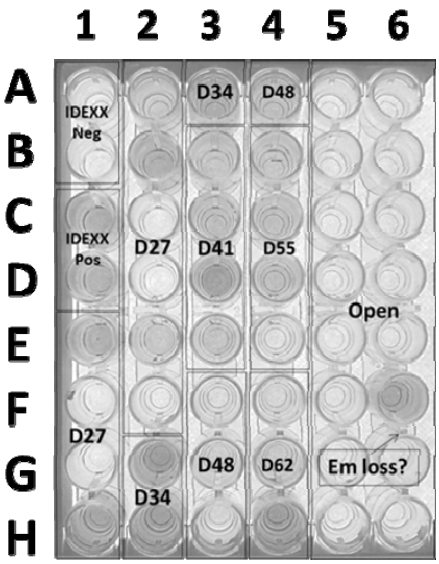

Figure 6. Results from an IDEXX milk pregnancy test. The milk test is based on PAG (the same placental protein that is tested when the blood test is performed). Results from the IDEXX milk test are shown to the left. Dark color indicates a positive test. The cows on d 27 after AI had not been pregnancy diagnosed at the time of the milk test. The cows tested in E1, H1, A2, B2, and E2 where subsequently diagnosed pregnant. The d 34, d 41, d 48, d 55, and d 62 cows were known to be pregnant. There were "open" cows in the pregnancy test as well. There was one open cow that tested positive. She was presumably undergoing embryonic loss.

The advent of reliable and affordable PAG assays for pregnancy testing in cattle creates options for early pregnancy diagnosis (25 to 30 days after insemination). Transrectal ultrasonography can be used within approximately the same time frame as the PAG test (Fricke, 2002). There are advantages of ultrasonography when compared with the PAG blood test. For example, ultrasound provides an instantaneous diagnosis of pregnancy and the ability to evaluate uterine and ovarian morphology of nonpregnant animals. It may also be possible to identify dead embryos and nonviable pregnancies when the ultrasound is used. These advantages must be weighed against the cost of ultrasound equipment, the technical skill required when performing the ultrasound procedure, and whether or not the farm has access to someone who can do early pregnancy diagnosis with ultrasound (Fricke, 2002).

Integrating pregnancy tests into a reproductive program

Pregnancy diagnosis from a blood sample enables the detection of nonpregnant (open) cows sooner after insemination. Commercially available blood PAG tests can reliably be performed at 28 days after insemination. We routinely perform PAG tests at 25 days after insemination (3 days earlier than the recommended minimum). In all likelihood, future tests for pregnancy will decrease further the interval between insemination and pregnancy detection. For example, if the ISGs are used then an interval as short as 18 to 20 days may be achievable (Figure 2). Shortening the interval between insemination and pregnancy detection enables a shorter interval between successive inseminations for herds performing synchronization and resynchronization without estrous detection. Giordano et al. (2013) concluded that a shorter interval between AI for non-pregnant cows yielded the greatest economic return regardless of the level of estrous detection. Using a chemical test for pregnancy may enable a shorter interval between AI.

A perceived downside of earlier pregnancy diagnosis is that some cows diagnosed pregnant will later be found open because pregnancies are lost over time when embryos die. Most cows are pregnant shortly after insemination but there are sequential periods of embryonic loss until the end of pregnancy. Most of the embryonic loss occurs before the placenta is fully formed at approximately 60 days after insemination (Santos et al., 2004). If cows are checked too early then a high percentage of the cows diagnosed as pregnant will later be found open because they have lost their pregnancies through the natural process of embryonic loss (Figure 7). If the cows are checked too late then open cows go undiagnosed for too long. In these open cows, earlier pregnancy detection could enable corrective intervention (for example resynchronization) so that they have an additional opportunity for a pregnancy to AI. In their economic analyses, Giordano et al. (2013) found that the advantage of using a chemical test to decrease the intervals between AI outweighed the potential disadvantage of diagnosing a pregnancy in a cow that will later undergo embryonic loss.

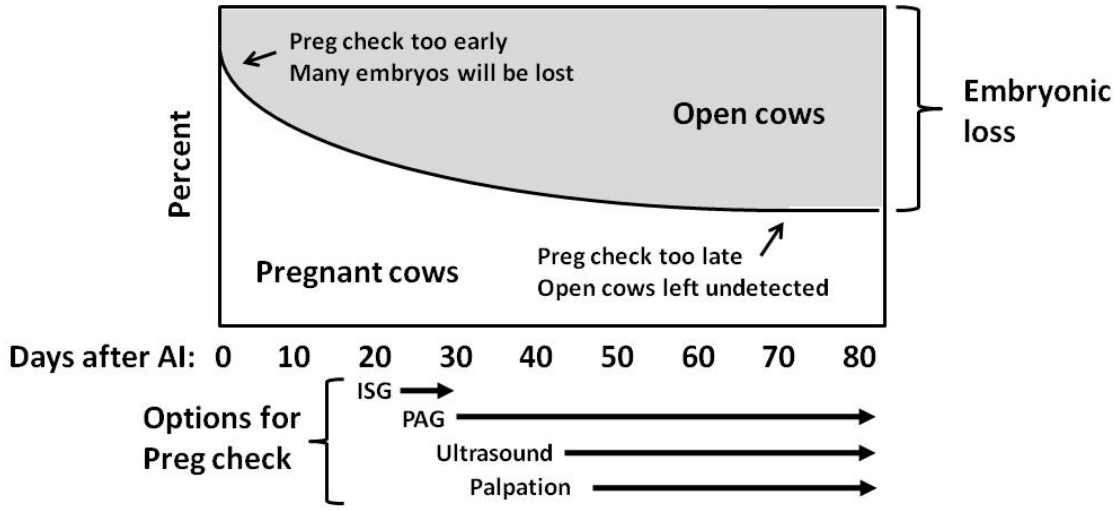

Figure 7**.** Conceptual diagram showing important considerations when making a decision about when to perform pregnancy diagnosis after an AI. A high percentage of cows are pregnant soon after insemination (top panel, left part of graph). The percentage of pregnant cows decreases over time because of the natural process of embryonic loss. If cows are pregnancy diagnosed ("Preg checked") too early then many cows that are correctly diagnosed as pregnant will lose their pregnancies during the embryonic loss period. The losses may diminish the value of the early diagnosis. If the preg check is scheduled too late (after most of the loss is completed) then an open cow may not be identified until too late.

Conclusions

Dairymen have options for pregnancy diagnosis. The traditional method of manual palpation is widely practiced. In some areas, ultrasound is performed so that more information is collected and pregnancies are detected sooner after insemination. In some geographical regions, there are too few skilled practitioners that can perform pregnancy diagnosis by manual palpation or ultrasound. In these places, blood and milk pregnancy tests for PAGs are a viable option for pregnancy diagnosis that can be used at any time after 28 days of pregnancy. The PAG tests are

based on well-understood physiology and are commercially available from at least three suppliers at competitive prices. There is also the possibility of ISG tests that could determine pregnancy by 18 to 20 days after insemination. If cows are checked too early, however, then a high percentage of the cows that are diagnosed as pregnant will later be found open because they have lost their pregnancies through the natural process of embryonic loss. An appropriate method for pregnancy diagnosis depends on the objectives of the reproductive program and considerations that are unique to each individual farm.

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The power of genomic evaluations for herd improvement.

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Introduction

Rapid developments in DNA-testing technology have led to dramatic changes in genetic improvement practices in the dairy industry in recent years. DNA marker information can now be used in genetic evaluations and this provides us with more accurate genetic information on young animals than what was previously available. Use of these methods has been widely applied by AI companies over the last five years which has led to more advanced genetics being provided by today's AI sires. Continued advances in DNA-testing technology have reduced the cost of DNA tests and there now may be opportunities for practical use of genomic evaluations in managing heifers and cows in commercial dairies.

The old adage of "mate the best to the best and hope for the best" is the general approach used in cattle breeding schemes. Since the 1970's, this practice was followed up with progeny testing of dairy AI sires to identify which individuals inherited a favorable set of genes from its parents. Once identified, these individual bulls were then made available to the industry for wide-scale AI use. This process takes over three years, is expensive and limits the number of animals that are "tested" to see if they inherited a favorable set of genes.

Today, the process begins the same way. But, with genomic evaluations, a DNA test can be run at birth to see if the resulting animal received a favorable set of genes from its parents. This allows AI companies to "test" hundreds of thousands of bulls rather than the couple of thousand bulls that previously had the opportunity to be progeny tested. This same approach, on a smaller scale, may provide some new opportunities to improve the management of herd replacements.

How do genomic evaluations work?

The cattle genome contains 30 chromosomes, 3 billion base pairs and 10,000+ genes. For decades, the dairy industry, through the DHI system, has been accumulating a large and powerful database of individual animal performance information. The sequencing of the bovine genome, published in 2006, directly led to the development of DNA chips that allowed affordable testing of a large number of markers. The dairy industry then invested in an effort to run these comprehensive DNA tests on a wide-range of historical bulls with completed progeny tests. The combination of comprehensive DNA information on a large number of reliably progeny-tested bulls provided the resource needed to identify associations between sections of chromosome and important performance traits that make genomic evaluations effective.

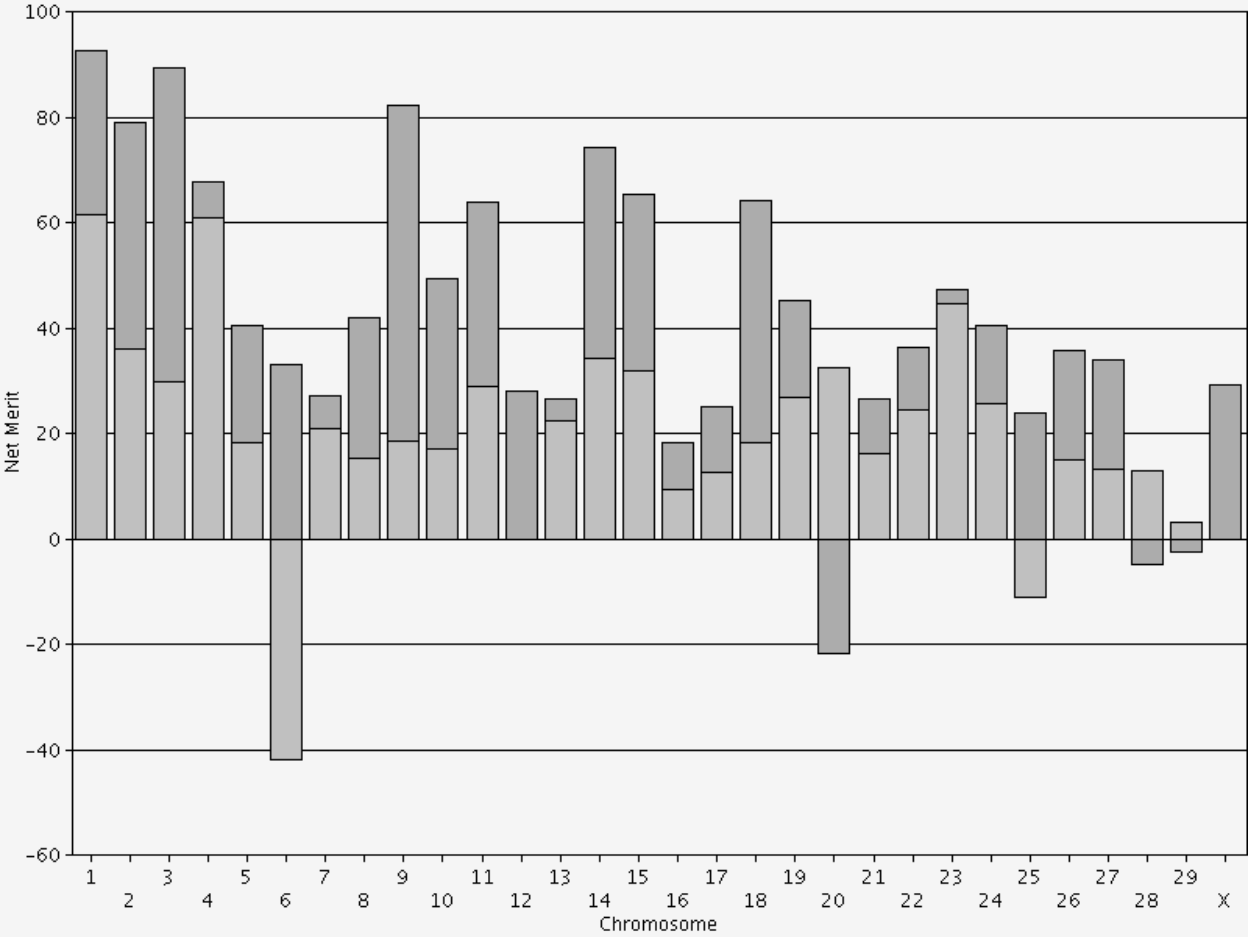
Table 1. Number animals in the April 2013 genomic evaluation reference population.

	Holstein	Jersey	Brown Swiss	Ayrshire
Progeny-tested bulls	21,904	2,855	5,381	639
Cows with lactation records	38,042	10,186	575	3

Genomic evaluations improve the accuracy of genetic evaluations in two ways. First, it helps us more accurately track the pedigree makeup of an individual. DNA testing allows us to identify the parents and grandparents with virtually 100% accuracy. In addition, there is no longer an assumption that an individual's DNA is equal 25% portions from each of its grandparent. The DNA information can be used to adjust these percentages based on the sections of chromosome the animal actually inherited. Knowing the contribution of the grandparents with more precision is helpful because the grandparent's genetic merit is often known with fairly high accuracy (especially the males).

The second way genomic evaluations improve accuracy is that with a large enough reference population the evaluations are able to identify relationships between sections of chromosomes and the traits that are evaluated. A lot of research has been done over the years to identify major genes and how they relate to economically important traits but, at this point, the genomic evaluations use none of that information. The methods rely on a dense set of DNA markers evenly scattered across all the chromosomes to capture a large percentage of the sequence variation that exists in our cattle. Then, with a large enough database, it can be determined how the parts of the chromosome affect the measured traits. Animals' evaluations are adjusted based on the sum of the chromosome effects it received from its parents.

Figure 1. Chromosome map for net merit dollars (NM$) of 7HO11351 Supersire.



Our current state of knowledge about individual genes and how different genes interact with each other is still very crude. Because of this, the accuracy of genomic evaluations is far from perfect. However, by using the DNA marker information available with current tests and using this to make better use of the large historical database of performance records that's available, the genetic merit of young animals is estimated more accurately than with traditional evaluations. Table 2 shows the accuracy improvement of genomic evaluations in heifers.

Table 2. Comparison of April 2013 genomic and traditional evaluations.

Trait	Breed	Genomic Reliability	Traditional Reliability	Difference	Daughter Equivalents
Milk	Holstein	71	28	+42	24.9
	Jersey	61	32	+29	11.3
	Brown Swiss	58	33	+25	9.2
Somatic Cell Score	Holstein	67	25	+42	54.5
	Jersey	54	27	+27	26.3
	Brown Swiss	54	29	+25	24.4
Daughter Pregnancy Rate	Holstein	62	22	+40	133.3
	Jersey	45	23	+22	51.8
	Brown Swiss	41	24	+17	37.6
Productive Life	Holstein	63	22	+41	70.7
	Jersey	47	23	+24	28.9
	Brown Swiss	49	26	+23	29.5
Overall Conformation	Holstein	70	25	+45	25.5
	Jersey	57	29	+28	11.4
	Brown Swiss	57	32	+25	10.8

One of the real benefits of genomic evaluations is that it provides more accurate genetic information on the health and fertility traits earlier in an animal's lifetime. For instance, with a trait like daughter pregnancy rate (DPR), without genomic evaluations, a bull would reach eight years old until 133 daughters-worth of DPR information was gathered. While the genomic evaluations for DPR are only 62% reliable, it provides enough genetic information on both males and females to make useful decisions when animals are in their prime reproductive years.

The values in Table 2 are theoretical estimates of improved accuracy based on the statistical assumptions used in the genomic evaluation model. USDA does historical analyses to check the assumptions and they adjust the assumptions as needed. It still is useful, though, to check whether the published values are delivering improved accuracy in practice. Table 3 compares the genomic predictions of AI sires when they were young calves to the actual yield differences seen in their daughters after they completed their progeny test.

Table 3. Genomic and traditional evaluations of AI sires compared to actual progeny milk production.

	No. of Bulls	Correlation Apr. '10 Genomic Prediction to Dec. '12 Dtr. Yield Deviation	Correlation Apr. '10 Parent Average Dec. '12 Dtr. Yield Deviation
Holstein	1,484	0.75	0.55
Jersey	305	0.69	0.57

The improvements in accuracy seen in practice don't quite match theoretical estimates. However, there are some key factors to keep in mind when making this comparison. The December 2012 progeny test results shown in Table 3 are based on a daughter group size of roughly 100 daughters. These are enough daughters to detect genetic differences between bulls but not nearly enough daughters to estimate the genetic merit of each bull with 100% accuracy. So, the standard of accuracy used in Table 3 may change as more daughter data on these bulls is gathered. Also, the accuracy values in Table 2 reflect the accuracy in today's genomic evaluations while the genomic evaluations used in Table 3 were from 2010 which may not have been as accurate. As animals with performance information and DNA test results are added to the database, the accuracy of the genomic evaluations gets incrementally more accurate. It is comforting to see that actual results experienced by AI companies show that genomic evaluations are significantly more accurate than the traditional genetic evaluations.

Table 4. Current genomic testing options

Chip Type	No. of Markers	Genomic Evaluation Reliability	Price
Low density	6,000 – 10,000	72%	$37 - $50
High density	50,000 – 80,000	75%	$75 - $125

With strong evidence showing that genomic evaluations deliver improved accuracy the challenge then becomes how should breeding programs be adjusted to best take advantage of this increased accuracy? Table 4 displays the current genomic testing options and their prices. Prices can vary depending on the size of the chip and the number of animals being tested. This is a rapidly developing area and we're likely to see a steady stream of testing options becoming available. Recent advances include expanding the low density chip to gain an improvement in genomic evaluation reliability. Also, several chips are available that combine genomic testing with testing for individual traits. Breeders can now get information about an animal's genotype for coat color, horned/polled and a variety of genetic defects at the same time as they are running a genomic test. The low density chip is used for almost all the testing done by commercial herds. The high density test is used for AI bulls and for elite females.

How this new tool should be used in a breeding program is a more difficult question. Let's follow the paths of selection, in their order of importance, to discuss the opportunities.

Sires of bulls

Because of the heavy use of AI in the dairy industry, this is the most powerful selection step in generating genetic improvement. Prior to the availability of genomic evaluations and the increasing attention given to health and fertility traits, the tendency of the industry was to use older bulls that had an established track record of transmitting both improved production as well as improved fitness traits as sires of the future AI bulls. This trend has shifted back to using younger sires as bull fathers because of the improved accuracy delivered by genomic evaluations. In fact, over 50% of today's AI bulls are results of matings to bulls that don't yet have progeny test results. Because of this the real evaluation accuracy of sire fathers is slightly lower today with genomic evaluations. But, the bulls with the best genetics are being used at much younger ages than previously. It is believed that this advance in generation interval will overcome the slight reduction in selection accuracy and the next generation of AI sires will be superior to today's offering. At this point, time will tell us whether this is an effective strategy and what is the best balance between generation interval and selection accuracy.

Dams of bulls

Genomic evaluations have delivered a big improvement in our capability to select the best cows to work with to produce the next generation of AI bulls. Traditionally we've relied on production records and classification scores to help us identify the cows with the best genetics. This process was complicated by the commercial reality that it was important to make sure cows with marketing potential also had good performance. Genomic evaluations provide better capabilities to separate out whether superior performance is due to genetics or management.

In addition, the affordability of DNA testing has expanded the pool of cows that are now eligible to become bull mothers. Geography was a key limiting factor for a sire analyst in tracking potential bull mothers prior to genomic evaluations. Genomic evaluations now provide a new common standard that makes it easier for breeders from all corners of the globe to establish credibility of their cows and get on AI companies' radar screens.

Many AI companies have taken a more hands-on approach in developing the cow families used in producing AI sires. Because of the improved accuracies of genomic evaluations, it is more valuable than ever to make sure that the heifers with the best genetics have full opportunity to generate pregnancies using advanced reproductive technologies. Use of in-vitro fertilization and embryo transfer in heifers has greatly expanded since the introduction of genomic evaluations. Many AI companies also own a herd of donor females so that they have more control and influence over the timing of embryo production and the mating combinations that occur in those critical early stages of the heifer's reproductive career.

Sires of cows

Due to the changes in AI Company practices mentioned above, the genetic level of AI sires available to dairy producers is higher than ever. The number one priority for dairy producers is to fine-tune their breeding practices and make sure they are maximizing the number of cows getting pregnant to AI sires. A variety of programmed breeding protocols, heat detection aids and activity monitoring systems are

available today that are very effective to assure the proper timing of AI breeding. Poor heat detection can no longer be used as an excuse not to use AI.

In addition, it is more costly than ever to feed a bull and it's important to keep in mind that dairy bulls are dangerous animals. Putting yourself, family members and employees in harm's way just doesn't make sense when there are a variety of tools available that make AI practical.

Today's dairy herd managers have access to a wider variety of AI sires than ever before. One area to assess is the producer's comfort level with using genomic-tested young sires at 70% reliability compared to progeny-tested bulls at 85% reliability. Key factors in this decision are semen price, size of the herd and the herd's breeding goals. Generally, the genomic-tested young sires will deliver better genetics on average but some individual bulls will not live up to their genomic prediction. For larger herds that can manage around some individual bull disappointments and are disciplined in using a variety of bulls without overusing individual bulls then genomic-tested young sires can be an effective option. If consistent and predictable performance is more critical than being on the leading edge of genetics, then using the more reliable progeny-tested sires may be a better option. There is no clear-cut answer on this question and the bottom line is that it comes down to producer preference.

The single most important on-farm management decision in guiding the genetics of a dairy herd is choosing which AI sires to use. With the improved accuracy delivered by genomic evaluations, producers are more likely to get what they select for. So, it's important to decide up front what traits are important to your herd and use that in deciding which bulls to use. Key factors in deciding which traits are important include how the producer is paid for the milk, what are the key limiting factors that are causing cows to leave the herd and what traits are needed for efficient operations of the milking herd. The list of traits should be comprehensive but not so long that it prevents meaningful progress in any one area. Focusing on five to ten priority traits is a reasonable target.

Once a list of priority traits is established, the most effective way to use this in selecting AI sires is to develop a selection index that includes these traits. There are a variety of websites and tools out there that can assist producers in developing a customized selection index. These tools will then help producers sort the list of currently available AI sires based on their own index. This will take a little time to set up initially but, once built, it becomes a very efficient way to sort through the mountain of available information every four months when the genomic evaluations are updated.

Dams of cows

The combination of improved reproductive performance, availability of sexed semen, high feed costs and high beef prices have caused dairy herd managers to look closer at being more selective in how they manage their heifers. Running a DNA test and obtaining an indication of the genetic capability of replacement heifers can be useful way to manage this valuable herd resource more efficiently. Developing a strategy of how to use this genetic information in making better management decisions before initiating the DNA testing is critical. Key items to review would include whether producers have plans for expanding, have surplus heifers and what options exist for marketing surplus calves.

One strategy producers might consider would be an aggressive genetic improvement approach. This strategy would include routine genomic testing of all heifer calves as they are born. As genetic information is gathered about the herd then the top 5% of animals would be used as embryo donors, the remainder of the top 25% would be bred to sexed semen, the middle two quartiles would be bred with conventional semen or be used as embryo recipients and the bottom 25% would be sold. This strategy would mean that more of the future replacements would come from the best members of the herd and would eliminate replacements coming from the bottom of the herd. This strategy would involve a fair amount of investment and is mainly for those herds that have a strong interest in generating a significant portion of their revenue from selling breeding stock.

Commercial operations may be more interested in minimizing the cost of raising replacements. The situation where dairies have surplus replacement heifers is much more common today than it was ten years ago. In this situation producers may want to consider a genomic testing strategy where replacements are screened based on the pedigree information that is available. The bottom 15% of heifers would be genomic tested with plans to sell the bottom 5% based on the resulting genomic evaluations. A general rule of thumb would be to test 3 animals for every one that you plan to sell.

An interesting strategy that is emerging is one where the herd is sorted based on their genetic merit and the top portion of the herd is bred to dairy bulls using a combination of sexed and conventional semen. The bottom portion of the herd is bred to beef bulls. This is a strategy seen more often in Jersey herds but is also may be an effective strategy for Holstein herds. Genomic testing a portion of the herd to help identify the ones to breed to beef bulls would help make the selection process more accurate.

Summary

1. Genomic evaluations are now available in Holsteins, Jerseys, Brown Swiss and Ayrshires that provide more accurate genetic information for young animals.
2. Improved accuracy of selection will accelerate genetic improvement for health and fertility traits particularly in Holsteins.
3. It’s more valuable than ever for producers to make full use of AI in their dairy herds.
4. Commercial animals can be genomic tested for $37 to $50.
5. Genomic testing can provide some new options for herds to more effectively manage their replacement heifers.

Key Performance Indicators for Improving Milk Quality

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Introduction

Increased involvement in the design and implementation of mastitis control programs is a potential growth area for many dairy veterinarians. As farms have expanded, the detection, diagnosis and administration of treatments for clinical mastitis has become the responsibility of farm workers. On many farms veterinarians are rarely consulted for mastitis unless an affected cow is near death. There are ample reasons for veterinarians to increase involvement in mastitis control programs. The occurrence of mastitis reduces milk production, increases the amount of milk discarded and increases premature culling and production costs.[1] Additionally, both clinical and subclinical mastitis have been demonstrated to reduce reproductive efficiency.[2,3] The prevalence of contagious pathogens has decreased as herds have modernized and adopted mastitis control practices.[4] Milk quality programs now tend to be focused on prevention of mastitis caused by environmental pathogens and other issues that influence consumer perceptions of milk quality. The purpose of this paper is to describe key performance indicators that dairy practitioners can use to monitor mastitis, milk quality and milking performance.

Defining and Detecting Clinical Mastitis

Clinical mastitis is technically defined as the production of abnormal milk with or without secondary symptoms but the working definition of clinical mastitis varies greatly among farm personnel. On large farms, detection of mastitis is usually dependent on the observational skills of the milking technicians. Veterinarians must actively communicate with milking technicians and farm managers to be sure that the definition of clinical mastitis and intensity of detection are consistent with farm goals. Mastitis case definitions should be simple and easily understood by all farm workers. Mastitis severity scores should be recorded in the permanent cow treatment records for each case.[5] Use of a 3-point severity scoring scale (1 = abnormal milk only; 2 = abnormal milk & abnormal udder; 3 = systemic symptoms) based on clinical symptoms is practical, simply recorded and can be an important way to assess detection intensity.[6,7] When using this scale, if the proportion of severe cases exceeds about 20% of all cases it is a signal that detection intensity and case definition should be investigated.

Monitoring clinical mastitis.

Animal health recording systems should consist of both temporary cow-side records (often used for day to day decision making) and permanent records (such as cow cards or computerized records) that are used to summarize trends over time.[8] The ideal system for recording clinical mastitis will allow the practitioner to evaluate important cow factors that define the probability of treatment success and to assess epidemiological trends.[9] To begin involvement in mastitis control programs, veterinarians should ensure that the following questions can be answered: 1) What is the incidence (rate of new cases) of clinical mastitis? 2) What proportion of cases are severe (severity score 3)? 3) What are the most common bacteria that are causing clinical mastitis? 4) What are the current treatment protocols? 5) How many days is milk discarded as a result of treatment? 6) How many cases: a) require changes to the original treatment protocol and b) experience recurrence of the case within

the same lactation? 7) What percent of lactating cows are being milked on less than 4 quarters? 8) What percent of cows that experience clinical mastitis are culled in the same lactation or die?

Practitioners who work with small herds, generally need to review data found in paper treatment diaries and will need to include data collected over longer time periods (3-4 month periods) to discern trends. For larger herds, computerized dairy management record systems can be configured to allow practitioners to rapidly review appropriate data.[8] Data entry should be structured to avoid redundancy, and only one mastitis event should be entered for each discrete case (defined at the cow level).[9] Researchers generally define separate cases of clinical mastitis based on an interval of 14-21 days between occurrences but this time period is not based on sound research and may be adapted to meet the needs of the farm. Key performance indicators that are defined at the cow-level (occurrence of mastitis in 1 or more quarters of a cow) rather than the individual quarter are easier to record and may better reflect the important economic consequences of mastitis (Table 1). Goals for key performance indicators (KPI) are derived from populations of herds and may need to be adjusted for individual herd circumstances.

Monitoring Subclinical Mastitis

Prevalence of mastitis is a function of incidence (development of new subclinical cases) and duration. For some herds, prevalence of subclinical mastitis may exceed goals even when relatively few new infections are occurring because of chronic infections caused by contagious pathogens. Alternatively, goals may be exceeded because of environmental mastitis problems that are characterized by high incidence of new infections of relatively short duration. The first step in monitoring subclinical mastitis is to ensure that SCC values are routinely obtained from all cows on a regular basis. Generally all cows with SCC values >200,000 cells/ml (linear somatic cell score of approximately 4.0) are considered to have subclinical mastitis. Assessments of subclinical mastitis should begin with the following questions: 1) What is the prevalence of subclinical mastitis (defined based on SCC)? 2) What is the incidence of subclinical mastitis (defined based on SCC)? 3) What are the most common bacteria recovered from cows with SCC values >200,000 cells/ml? 4) What proportion of subclinical cases are chronic (persist more than 2 months)? 5) What is the prevalence of subclinical mastitis by days in milk and parity? 6) What proportion of cows have subclinical mastitis at the first test and the last test? Data to answer these questions can often be found in summarized reports available from DHIA testing centers or the data can be downloaded and manipulated in customized spreadsheets or dairy management programs. Common KPI for subclinical mastitis are: 85% cows with somatic cell counts $\leq$200,000 (prevalence) and less than *<5% of cows developing new subclinical mastitis infections per month* (incidence) (Table 2).

Measuring and monitoring bacteriological quality of bulk milk.

Many processors measure bacteriological quality of milk on every tanker load of milk and provide online access to daily milk quality reports. Bacteriological contamination of raw milk can occur from 2 basic sources: 1) organisms can contaminate milk from environmental sources (especially contamination during the milking process) or 2) via mastitis organisms from within the udder.[10] Raw milk from healthy udders normally contains < 1,000 total bacteria per ml; and therefore do not significantly contribute to total numbers of

microorganisms in bulk milk, or to a potential increase in bacterial numbers during refrigerated storage.[11] It is unusual for mastitis to contribute to increased total bacteriological counts in raw milk but occasionally cows with mastitis can shed large numbers of microorganisms. Investigations of bacteriological quality of raw milk begin with the following questions: 1) How many tests of bacteriological quality have been performed and do the counts demonstrate a trend or a "spike?" 2) What is the average, minimum and maximum standard plate count? 3) What other diagnostic tests of milk quality have been performed and how do they compare?[12] 4) If available, what are the values for: a) laboratory pasteurized count (LPC); b) preliminary incubated count (PIC); and c) coliform count (CC)? The SPC is an overall measure of milk quality but a single SPC value is not very useful diagnostically. Consistently increased values for SPC are an indication of a milk quality problem and the best diagnostic strategy is to perform strategic sampling of milk at various points throughout the milking process. Comparison among the values of diagnostic counts (SPC, LPC, Coliform count, and SCC) can give valuable clues as to the likely source of the problem.[10] The LPC is basically a SPC performed on milk that has been heated to 145F (62.8C) and held for 30 minutes (low temperature-long time pasteurization). The objective of performing the LPC is to identify organisms that survive pasteurization (thermoduric bacteria). Typical mastitis causing organisms do not survive pasteurization. Thermoduric bacteria may include Micrococcus, Microbacterium, Lactobacillus, Bacillus, Clostridium and occasional Streptococci. Increased LPC are often associated with the development of biofilms on unclean equipment. The LPC should be less than 100 to 200 cfu/ml and a LPC below 10 cfu/ml indicates excellent equipment hygiene.[10,12] Goals for high performing herds are set by processors and are not uniform across the industry but SPC of <5,000 cfu/ml and LPC of < 200 cfu/ml are reasonable goals for high performing herd (Table 3).

Conclusion

The delivery of milk quality programs by veterinarians is an important overall component of a dairy production medicine program. Preventing mastitis and improving milk quality is vitally important role that contributes to improved animal wellbeing, enhanced farm profitability and better assurances that food is being produced in a safe and sustainable way. Dairy veterinarians should seek out involvement in continuing education programs that focus on research based methods and advancements in mastitis control. Milk quality programs must continue to advance with changes in pathogens, changes in milking equipment and cow housing systems and as societal expectations evolve.

Table 1. Calculation of suggested key performance indicators for clinical mastitis. For ease of interpretation, a case is defined as the occurrence of mastitis in 1 or more quarters of a cow.

Indicator	Calculation[a]	Suggested Goal
Incidence Rate	Sum of first cases occurring in the appropriate time period[a] divided by average number of lactating cows in the same time period[b]	< 25 new cases per 100 cows per year (about 2-3 cases per 100 cows per month)
Proportion of cases scored 3 (severe)	Number of severity score 3 cases occurring divided by the total number of cases occurring	5-20% of total cases
Proportion of cases that die	Number of cows experiencing mastitis cases that resulted in death divided by the total number of cows experiencing mastitis	2%
Proportion of cases requiring treatment changes	Number of cases where the initial protocol is changed or supplemented because of non-response divided by the total number of detected cases[c]	<20%
Proportion of cases that are recurrent (second or greater treatment)	Number of cows with second or greater case of mastitis occurring >14 days post treatment divided by the total number of cases of mastitis	<30%
Proportion of cows with > 1 quarter affected	Number of cases with 2+ quarters affected divided by the total number of cases	<20%
Number of days milk discarded (per case)	Sum of the number of discard days for the time period divided by the total number of cases	4-6 days (unless many cows are receiving extended therapy because of a high prevalence of Staph aureus)
Percent of herd milking with <4 quarters	Number of cows milking with < 4 quarters[d] divided by the number of lactating cows	<5%

[a]numerators and denominators should include the statement "in the appropriate time period." The appropriate time period will vary depending on herd size.;[b] a more correct denominator would exclude cows that had previously experienced a clinical case within that lactation; [c]cases which are detected but do not receive initial antimicrobial treatments should be included in this calculation; [d]herds that use quarter milkers to discard milk from selected quarters should include those cows in the numerator

Table 2. Calculation of suggested key performance indicators for subclinical mastitis.

Indicator	Calculation	Suggested Goal
Prevalence	Number of cows with SCC >linear score 4[a] divided by the number of cows with somatic cell counts	<15% of the herd
Incidence	Number of cows with SCC > linear score 4[a] for the first time in the time period of interest[b] divided by the number of cows with SCC below the threshold in the previous time period	<5% if incidence is determined based on the first SCC above threshold in the lactation; up to 8% if calculated based on month to month changes in SCC[b]
Prevalence at 1st DHIA Test	Number of cows with SCC >linear score 4[a] at the 1st DHIA test divided by the number of cows with first test DHIA somatic cell counts	<5% of 1st lactation <10% of lactation 2+
Prevalence at last DHIA Test before dry off	Number of cows with SCC ≥linear score 4[a] at the last DHIA test before dry off of the lactation divided by the number of cows with last test DHIA somatic cell counts	<30% of cows with last test days before dry off

[a]for the purpose of herd monitoring, linear somatic cell score of 4 is used interchangeably with somatic cell count of >200,000 cells/ml; [b]The appropriate time period will vary depending the intended use of this index. Many DHIA centers & computer management programs will calculate this index based on changes between 2 months. Others may calculate it based on the SCC values available in the current lactation.

Table 3. Key performance indicators and sources of typical bacteria used to troubleshoot problems with bacteriological quality of raw bulk milk.

Indicator	Type of Bacteria Detected	Common Sources	Suggested Goal
Standard Plate Count	Quantifies most viable, aerobic bacteria found in milk	Contamination during milking; problems with milk cooling; cleaning failures	<10,000 cfu/ml
Laboratory pasteurized count	Thermoduric bacteria (such as bacillus, clostridia etc.)	Biofilm development on milking equipment as a result of cleaning failures; occasional problems with contamination	<200 cfu/ml
Preliminary incubated count	Psychrotrophs (such as pseudomonas and others)	Contamination during milking; cooling problems	<10,000 cfu/ml
Coliform count	Coliform bacteria (such as E.coli and Klebsiella)	Contamination during milking rarely mastitis	<100 cfu/ml)

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Patho-biology and Epidemiology of Gram-Negative Bacteria in Bovine Mastitis

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ABSTRACT

Mastitis due to Gram-negative bacteria is a common occurrence in dairy farms with a low bulk somatic cell count. All Gram-negative bacteria have lipopolysacharides as a major part of their outer membrane. The biological activity of LPS resides predominantly within the lipid A fraction that anchors LPS in the bacterial outer membrane. *E. coli* and particularly Klebsiella spp. intramammary infections cause a severe inflammatory response, while Serratia spp. intramammary infections are usually associated with less severe clinical signs. Gram-negative intramammary infections are often environmental in nature but may occur in within farm clonal outbreaks, suggesting contagious transmission. Severity of infections with Gram-negative bacteria may be reduced by vaccination with a core antigen vaccine. Treatment of coliform intramammary infections may result in an important increase in bacterial cure compared to untreated controls.

Introduction

Gram-negative bacteria are an important cause of bovine mastitis throughout the world. With the advance of our understanding of the main risk factors for classical contagious bacteria such *Streptococcus agalactiae* and *Staphylococcus aureus*, we have observed a decrease in the prevalence of these two mastitis pathogens. However, this decrease has gone hand in hand with an increase in the incidence of intramammary infections (IMI) due to Gram-negative bacteria (Barkema et al. 1998, Olde Riekerink et al. 2008). Gram-negative bacteria, mostly coliforms (*E. coli*, *Klebsiella* spp. and *Enterobacter* spp.), cause 40% of all clinical mastitis (CM) cases (Erskine et al., 1988; OldeRiekerink et al. 2008) and up to 25% of cows in well-managed herds are annually diagnosed with CM caused by coliforms (Erskine et al., 1988). The most common coliform species are *Escherichia coli* and *Klebsiella* spp. (Todhunter et al. 1991. Barkema et al. 1998, OldeRiekerink et al. 2008). The incidence of CM arising from gram-negative bacterial infection is inversely related to bulk milk SCC (Barkema et al., 1998), economic losses attributed to intramammary gram-negative infection can therefore be expected to increase as dairymen continue to strive for lower bulk milk SCC. Here we present a discussion on Gram-negative bacteria, their bacterial characteristics and immune response patterns. Then we will discuss some new findings with regard to Gram-negative mastitis causing bacteria.

Gram-negative bacteria

Enterobacteriaceae

Some of the more common clinically important genera of the family Enterobacteriaceae include: Salmonella, Shigella, Proteus, Escherichia, Citrobacter, Enterobacter, Serratia, Klebsiella, Morganella, Yersinia, Edwardsiella, Providencia. These genera include recognized mastitis pathogens such as *E. coli*, and *Klebsiella* spp. but also the less well know but known mastitis causing organisms such as *Raoutella* spp., *Pseudomonas* spp, *Enterobacter* spp., *Shigella* spp *and Citrobacter* spp. The Enterobacteriaceae are rod shaped, are all gram-negative and are typically diagnosed in the laboratory with the use of the MacConkey agar plates (NMC 1999). Lactose fermenters such as *E. coli*, Enterobacter and Klebsiella will produce acid, which lowers the pH of the MacConkey agar below 6.8 and results in the appearance of red/pink colonies. Some organisms ferment lactose slowly or weakly. These include *Serratia* spp. and *Citrobacter* spp. (NMC 1999). One of the several unique characteristics of Gram-negative bacteria is the structure of the outer membrane. The outer membrane consists of a complex lipopolysaccharide (LPS) whose lipid portion may act as an endotoxin. This lipopolysacharide structure is present in all Gram-negative bacteria but is structurally different between and within Gram-negative bacterial species (Caroff et al. 2002).

Lipopolysacharides

Bacterial lipopolysaccharides (LPSs) are the major component of the outer membrane of Gram-negative bacteria. They have a structural role since they contribute to the cellular rigidity by increasing the strength of cell wall (De Castro et al. 2010). The LPS structure also mediates contacts with the external environment and will allow different species to live under different environmental conditions. The low permeability of the outer membrane acts as a barrier to protect bacteria from a number of environmental stressors, including antimicrobial compounds. The lipopolysaccharides also have an important role in the activation of the host innate immunity (De Castro et al. 2011,). For that reason, LPS are considered pathogen-associated molecular patterns (Schukken et al. 2011). The LPSs are macromolecules generally with three defined components: the lipid A fraction, the inner and out core oligosaccharide and finally a polysaccharide portion, the O-chain (Caroff and Karibian 2003). In some Gram-negative bacterial strains, there is no O-chain and these strains are identified as 'rough-types' as opposed to the 'smooth types' when the O-chain is present. The O-chains are in direct contact with the host during infection and form the basis for serotype classification (Caroff and Karibian 2003). Figure 1 shows a schematic representation of the Gram-negative cell wall. Although LPS is not actively secreted by the bacterial cells, small amounts of the LPS are released into the bacterial environment under circumstances such as cell division. Larger amounts are released when the bacteria are killed by antibiotics, phagocytosis, or the complement complex.

The role of LPS in the activation of the host immune response is dose dependent. Small amounts of LPS can be used as a protective compound by stimulating the immune system (Petzl et al. 2011, Gunther et al., 2012). Large amounts of LPS, however, induce high fever, increase heart rate, and lead to septic shock and death by lung and kidney failure, intravascular coagulation, and systemic inflammatory response (Caroff and Karibian 2003). The biological activity of LPS resides predominantly within the lipid A fraction that anchors LPS in the bacterial outer membrane. Lipid A differs between species in acyl-side chain number and length; however, the overall structure and synthetic pathway is conserved between gram-negative bacteria. The lipid A structure of *E. coli* consisting of six acyl chains and two phosphate groups and is one of the most potent stimulators of the innate immune system through it binding to Toll-like receptor 4 (TLR-4) in a tight connection to the LPS binding protein (Schukken et al. 2011). Deleterious effects of LPS on the mammalian host, such as fever, inflammation, acute phase response, and multiple organ failure, are generally attributed to lipid A fraction of LPS (Caroff et al. 2002). It has been suggested that the variability in lipid A structure between bacterial species explains the difference in the biological activity of LPS (Caroff et al. 2002). Changing the number of acyl groups from six to five decreases the biological activity of LPS approximately 100-fold. With regard to the main mastitis pathogens, *E. coli* has six acyl groups, whereas *Serratia marcescens* has five acyl groups and *Klebsiella pneumonia* has been reported to have 7 acyl groups (Llobet et al. 2011). This difference in the structure of the lipid A portion of LPS may partly explain the difference in immune response patterns and clinical presentation that is observed between these three important mastitis pathogens.

Immune response patterns

The innate immune response is characterized by the rapid activation of antimicrobial host defense mechanisms in the mammary gland of the cow (Schukken et al. 2011). The ability to respond to a large number of pathogens is possible through evolutionary conserved pattern recognition receptors. These receptors are capable of recognizing molecular patterns that are shared by bacterial pathogens. Toll-like receptor 4, which is expressed on a wide array of cell types including macrophages, neutrophils, and epithelial cells, is one such pattern recognition receptor (Bannerman 2009). The TLS-4 receptor recognizes bacterial LPS, particularly the lipid A fraction of LPS. Activation of TLR-4 and other pattern recognition receptors leads to the generation of an inflammatory response that is modulated, in part, by cytokine production (Schukken et al. 2011). In a series of experimental IMIs, Bannerman and co-workers provided a unique insight in the comparative activation of the innate immune response after a challenge with a number of Gram-negative bacterial species (Bannerman et al. 2004a,b,c). Since these scientists were using similar methodologies throughout the challenge trials, it is now possible to compare the innate immune response of the host to IMIs with these Gram-negative bacteria. Figure 2 shows the response of the challenged cows to an experimental IMI with *E. coli, Klebsiella pneumonia and Serratia marcescens*. The results in this figure make it very clear that the innate response between these three pathogens differs sharply, with the most sever response observed after *Klebsiella pneumoniae* IMI and the more modest response observed after an IMI with *Serratia. marcescens*. This difference in response was apparent in both the pro-inflammatory response as shown by the concentration of TNF-α (Figure 2, top) as well as in the regulatory immune response as shown by the concentrations of the cytokine IL-10 (Figure 2, bottom). Although the precise mechanism of the difference in innate immune response are not fully clear, the functional form of LPS in these three organisms is likely to play a role in the observed pathogenicity.

E. coli and Klebsiella spp. Mastitis

Differences in the pathogenicity of *E. coli, Klebsiella* spp. and *Serratia* spp. as mastitis pathogens have been noted (Bannerman 2009, Grohn et al. 2005). These differences include a longer duration of infection for *Klebsiella* spp. and *Serratia* spp compared to *E. coli* (Todhunter et al., 1991). Also, *Klebsiella* spp. IMIs appear to be more severe than *E.coli* IMIs and *Serratia spp*. IMI appear to be less severe compared to *E. coli* IMIs. Severity of clinical episodes, poor response to vaccination, and the paucity of effective treatments make Klebsiella mastitis especially troublesome when compared with *E. coli* (Erskine et al., 2002; Roberson et al., 2004). The severity of clinical mastitis due to coliforms was highlighted in a series of papers from our research team. In a paper on the effect of pathogen specific clinical mastitis on milk production of CM affected cows (Grohn et al. 2004), a difference in milk production loss between *E. coli* and *Klebsiella* spp. IMI became clear (Figure 3, top). The duration of milk production loss was substantially longer in cases of *Klebsiella spp*. compared to clinical cases due to *E. coli*.

Similarly, the risk of culling (Grohn et al. 2005) after a case of clinical mastitis was substantially larger in cases of *Klebsiella* spp mastitis compared to *E. coli* clinical mastitis (Figure 3, bottom). These findings were in line with observations of others where Klebsiella CM turned out to be more severe compared to *E. coli* mastitis (Erskine 2002, Roberson et al. 2004).

Epidemiology

Classically, coliform IMIs are considered to be of environmental origin and improving environmental hygiene and optimizing the cow's immune response would prevent therefore aid in the prevention of these infections. Environmental infections are considered to be not contagious, and the practical implication of that is that every IMI would be associated with its own bacterial strain (Paulin-Curlee et al. 2008). This is in stark contrast to contagious infections, where many IMIs share the same strain (or clone) of a bacterial species (Zadoks and Schukken, 2008). With the advance of molecular diagnostic techniques, the differentiation between clonal and non-clonal outbreaks of intramammary infections on dairy farms has become possible for routine outbreak evaluations. Recently, Munoz *et al.* (2007) described the occurrence of two *Klebsiella* spp. mastitis outbreaks on a single dairy farm. Klebsiella isolates from milk, feces, and environmental sources were compared using random amplified polymorphic DNA (RAPD). The first mastitis outbreak on the described farm was caused by a single strain of *Klebsiella pneumoniae*, which was detected in milk from eight cows. In figure 4, an example of a clonal *K. pneumoniae* outbreak on a New York dairy farm is shown. This RAPD type was also isolated from the rubber liners of milking machine units after milking of infected cows and from bedding in the outbreak pen. This observed predominance of a single strain would indicate contagious transmission of the organism or exposure of multiple cows to an environmental point source. When the authors implemented intervention methods that targeted the prevention of transmission via the milking machine as well as improvement of environmental hygiene, no new cases with the initial RAPD type were observed (see also figure 6). A second outbreak of Klebsiella mastitis that occurred several months later on the same farm was caused by multiple RAPD types, which rules out contagious transmission and indicates infections originating from the environment (Munoz *et al.* 2007). Using the RAPD technique has shown to be useful in distinguishing clonal versus non-clonal outbreaks across several Gram-negative mastitis pathogens (Zadoks et al. 2011).

Vaccination and Treatment

A mutant Escherichia coli O111:B4 known as J5 (Teng et al. 1985) has been used for the development of a bacterin to reduce mastitis severity due to coliform organisms. This mutant is deficient in the enzyme uridine 5'-diphosphate-galactose 4-epimerase so that it cannot attach the 0 side chains of LPS (see figure 1). Without the O side chains, the core oligosaccharide with the bound lipid A becomes exposed and can stimulate antibody response by the host. The induced antibodies may react with the core region and lipid A of all LPSs, regardless of bacterial species. Commercial vaccines developed against the J5 core antigen of coliform bacteria have been in use for approximately 15 years (González et al., 1989). Reduction of coliform CM in vaccinates

compared with controls has been reported (González et al., 1989). However, more often, J5 vaccination is associated with a reduction in severity and improved herd survival (González et al., 1989, Wilson et al. 2007). Wilson et al. (2008) showed that the milk loss due to clinical mastitis was substantially less among J5 vaccinates compared to controls; however, this protective effect of vaccination waned with increasing time since the last vaccination. It was also reported that J5 vaccination was also associated with survival advantages after a case of clinical coliform mastitis (Wilson et al. 2007). Particularly, Wilson et al. 2007 reported that vaccinates with *Klebsiella* CM were less likely to be culled for mastitis compared to unvaccinated controls. Together these data indicate that J5 vaccination is one of the tools available to reduce losses due to clinical coliform mastitis.

Studies reporting on treatment efficacy of antibiotic treatment of Gram-negative mastitis have generally shown a very limited efficacy of antibiotic treatment (Roberson et al. 2004). A field study of naturally occurring severe CM caused by coliform organisms showed that intramuscular treatment with ceftiofur reduced the risk of death or culling (Erskine et al., 2002). A proportion of approximately 50% cure, was observed after intramuscular ceftiofur treatment of 8 animals with severe CM, while cure in controls animals was approximately 25% (Erskine *et al.*, 2002). In a recent study on mild and moderate clinical coliform mastitis in six large dairy farms, it was reported that across farms and coliform species, 5-day treatment with intramammary ceftiofur resulted in a significantly higher probability of cure compared to no treatment (Figure 5, Schukken et al. 2011). Across herds and bacteriological species, bacteriological cure was 73% in the treated animals and 38% in control animals. Although treatment of Gram-negative mastitis is not as successful as treatment of most Gram-positive bacteria, the availability of third generation cephalosporins for clinical mastitis treatments appears to provide an important tool for the treatment of clinical mastitis caused by this organism. An important benefit of successful treatment of Gram-negative bacteria is the reduction in the duration of infection. Shorter duration infections will have a lower probability of transmission to other cows (Figure 6). Successful treatment is therefore not only of benefit to the cured cow, but also to the herd (Halasa et al. 2012).

Prevention

Identification of potential sources of *E. coli* and Klebsiella is important for implementation of preventive measures that decrease exposure and limit the risk of udder infections. Bedding materials can be important sources for both *E. coli and* Klebsiella organisms on dairy farms (Munoz et al. 2006). Furthermore, fecal shedding of these bacteria by healthy dairy cows has been documented recently (Munoz and Zadoks, 2007). Consequently, feces and manure also constitute sources of exposure for dairy cows (Munoz et al., 2007, Verbist et al. 2011). Direct contact of the teat ends with materials that contain coliforms, such as bedding, feces, manure splash, water, milk, mattresses, legs or liners may provide the bacteria with access to the udder (Zadoks et al. 2011), which may ultimately result in IMI and subsequent CM. Dirty udders were a significant risk factor for presence of Klebsiella after udder preparation (Munoz et al. 2006). In summary, prevention of coliform IMI appears to be possible through careful monitoring and

improving of hygiene in the environment of the cow.
Another component of prevention is improvement of cow susceptibility to intramammary infection. There are a number of factors that affect the cow's susceptibility. This includes the quality of the innate immunity, such as teat-end quality, nutritional status and genetic ability to combat infections. Important nutritional components include minerals such as Selenium and Copper and Vitamins such as Vitamin E, finally the presence of a negative energy balance puts a cow at risk for more severe inflammatory responses to a coliform infection.

Discussion and Conclusions

Mastitis by Gram-negative infections is of increasing importance on modern and well-managed dairy farms. Without a doubt, *E. coli* tends to be the most important cause of these Gram-negative infections when the data are tallied across farms (Barkema et al. 1998). However, more precise investigation of individual farms often reveals a farm-specific infection pattern where a single Gram-negative bacterial species predominates. Here we have shown the presence of outbreaks on individual dairy farms with *Klebsiella pneumoniae*. It is quite surprising to identify the difference in host immune response pattern and the associated clinical and subclinical presentation of intramammary infections due to the different Gram-negative organisms. Experimental and field observations would suggest that among the Gram-negative bacterial causes of mastitis, *Klebsiella* spp. are causing the most severe cases, closely followed by *E. coli* and then much less clinical severity is observed in *Serratia* spp. and Enterobacter spp cases. The precise mechanisms that would explain the difference in clinical severity are not known, but the most likely explanation appears to be the structure of the lipid A fraction of the LPS of the bacterial species. Important differences in the lipid A fraction of LPS between and within bacterial species are observed.
Prevention of IMIs with Gram-negative bacteria has components that are generic across species and components that are species specific. Generic prevention may be obtained by improving hygiene and reducing exposure of teat ends to environmental contamination. Also the use of a J5 bacterin is expected to provide some reduction in severity of Gram-negative IMIs across bacterial species. Specific prevention programs will depend on the actual transmission behavior of the dominant species causing IMIs in the herd. Several clonal outbreaks of Gram-negative bacterial species have been described. In such situations, optimal milking procedures, segregation and culling of infected animals and targeted treatment would be advisable.
Antimicrobial treatment of Gram-negative bacteria has often considered to be of limited value and treatment should be more targeted towards cow survival and reduction of clinical symptoms. More recently, extended treatment with a third generation cephalosporin was reported to be efficacious in the treatment of *E. coli* and *Klebsiella spp.*, but not of *Enterobacter cloacae*. Further investigations in effective treatment protocols for Gram-negative IMIs are warranted.
A herd characterized by a stable healthy udder health status will need to have three components of management in excellent working order (figure 6). These three components are 1) a low risk of new IMI, 2) a short duration of IMI that are occurring and 3) a low risk of on-farm infection

transmission. These three components are important irrespective of the causal organisms and will guarantee that the herd is not in a situation characterized by either a contagious or environmental transmission pattern.

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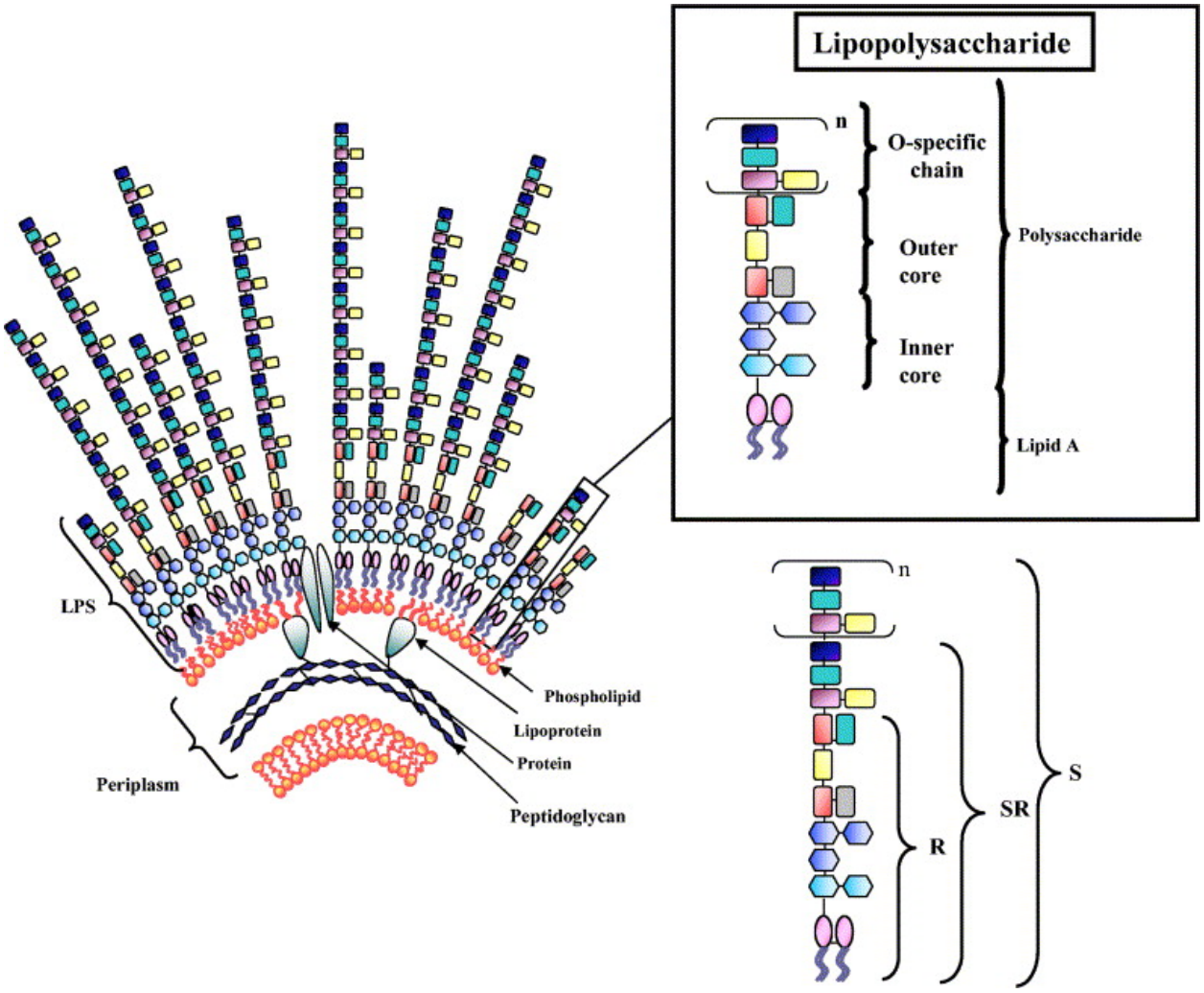


Figure 1. Schematic representations of the enterobacterial Gram-negative cell wall(left), a lipopolysaccharide structure (right), R, SR, and S indicate the structures of rough-type, semi-rough type (with only one *O*-chain subunit) and smooth-type lipopolysaccharides, respectively. Reproduced with permission from Caroff and Karibian (2003). Copyright 2003 by Elsevier Ltd. All rights reserved.

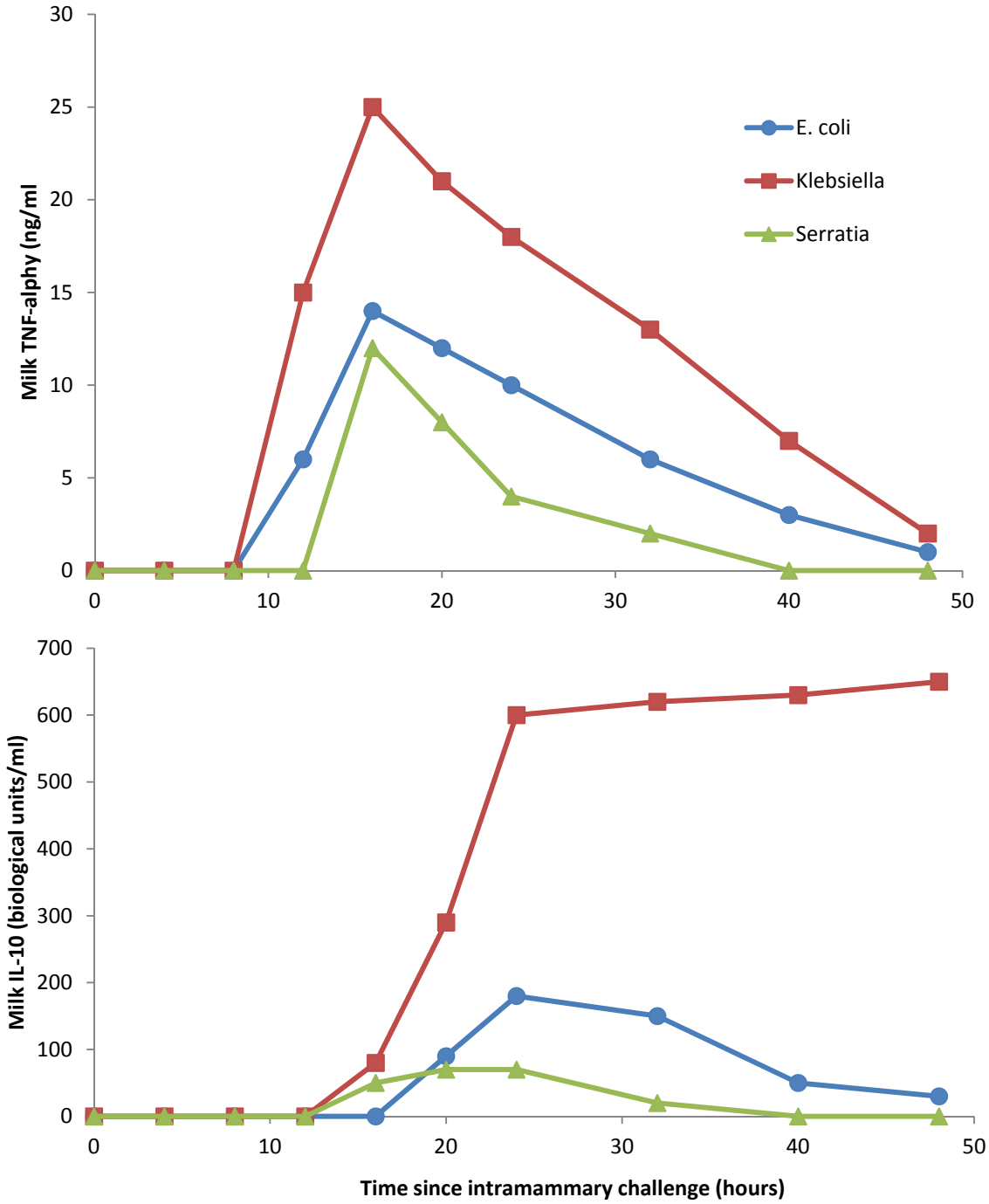


Figure 2. Cytokine profiles after experimental challenge infections with three different Gram-negative bacterial species. In the top graph, milk Tumor Necrosis Factor – alpha (TNF-α) concentrations (in ng/ml) and in the bottom graph milk IL-10 concentrations after intramammary challenge infection with *E. coli, K. pneumonia* and *S. marcescens* are shown. Data from Bannerman 2009.

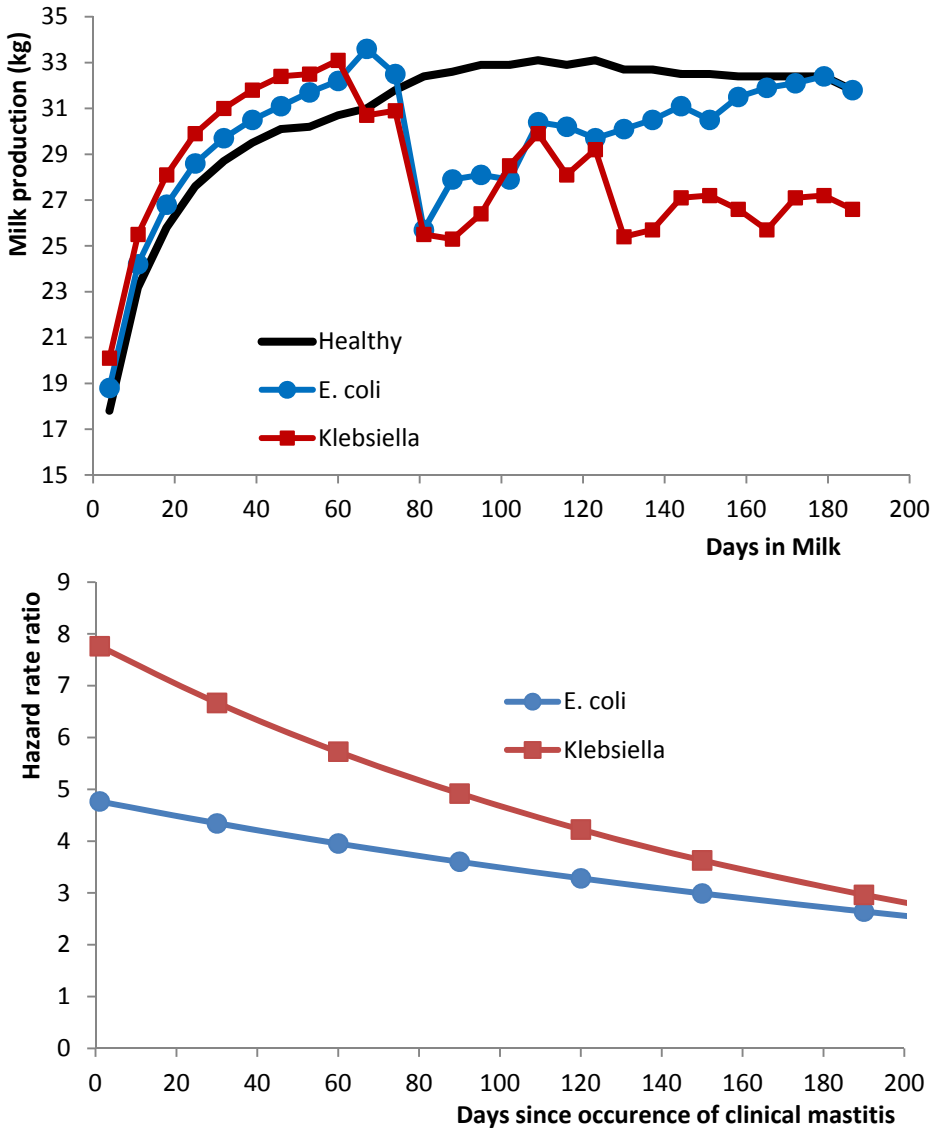


Figure 3. Milk production effects (top) of an *E. coli* and a *Klebsiella spp*. case of clinical mastitis compared to milk production of healthy herd mates. The milk production loss pattern in *Klebsiella spp*. indicates an earlier onset and a longer duration of milk production loss compared to E. coli, whereas the severity of loss (indicated by the nadir) is approximately similar (data from Grohn et al. 2004). Hazard rate ration of cows with a case of E. coli or *Klebsiella spp*. in early lactation (data from Grohn et al. 2005).

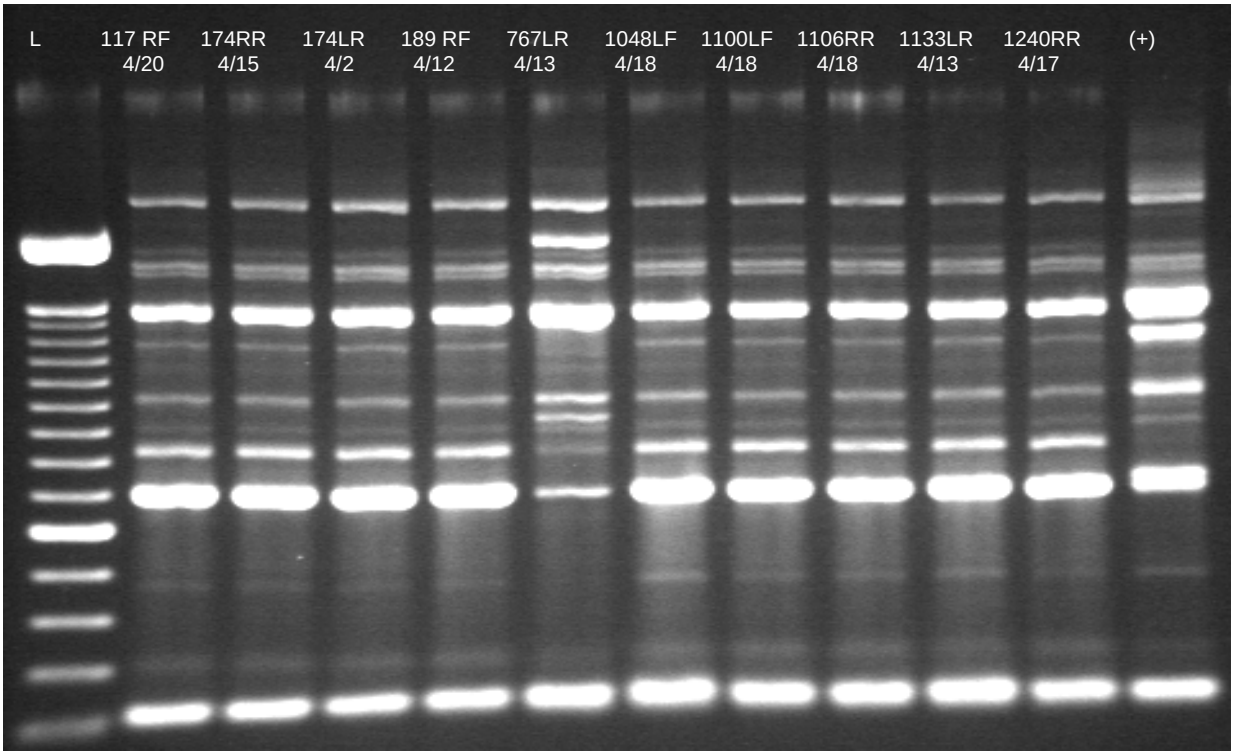


Figure 4. An electrophoresis gel depicting the result of an RAPD analysis of the DNA of *K. pneumoniae* isolates from 10 cases of clinical mastitis on a single dairy farm. The text above the gels indicate the cow number, quarter and date of the clinical case, (+) and (-) for positive and negative controls, W for a water control and L for DNA ladder. The figure shows that 9 out 10 clinical cases were caused by the same clone of *K. pneumoniae*.

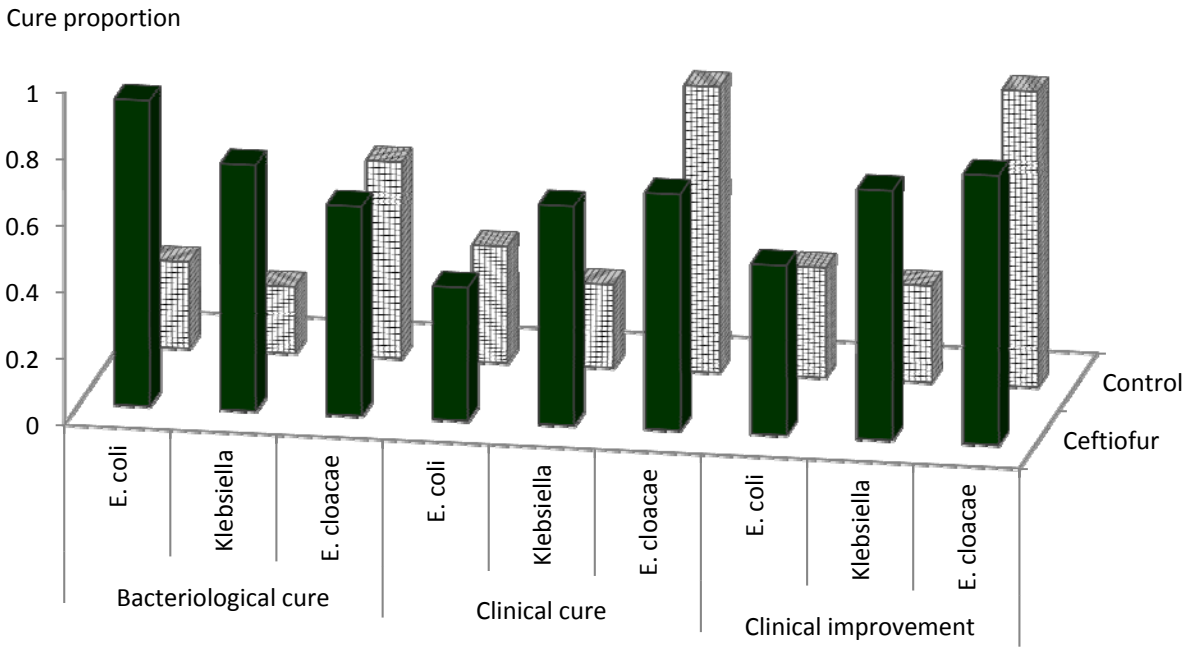
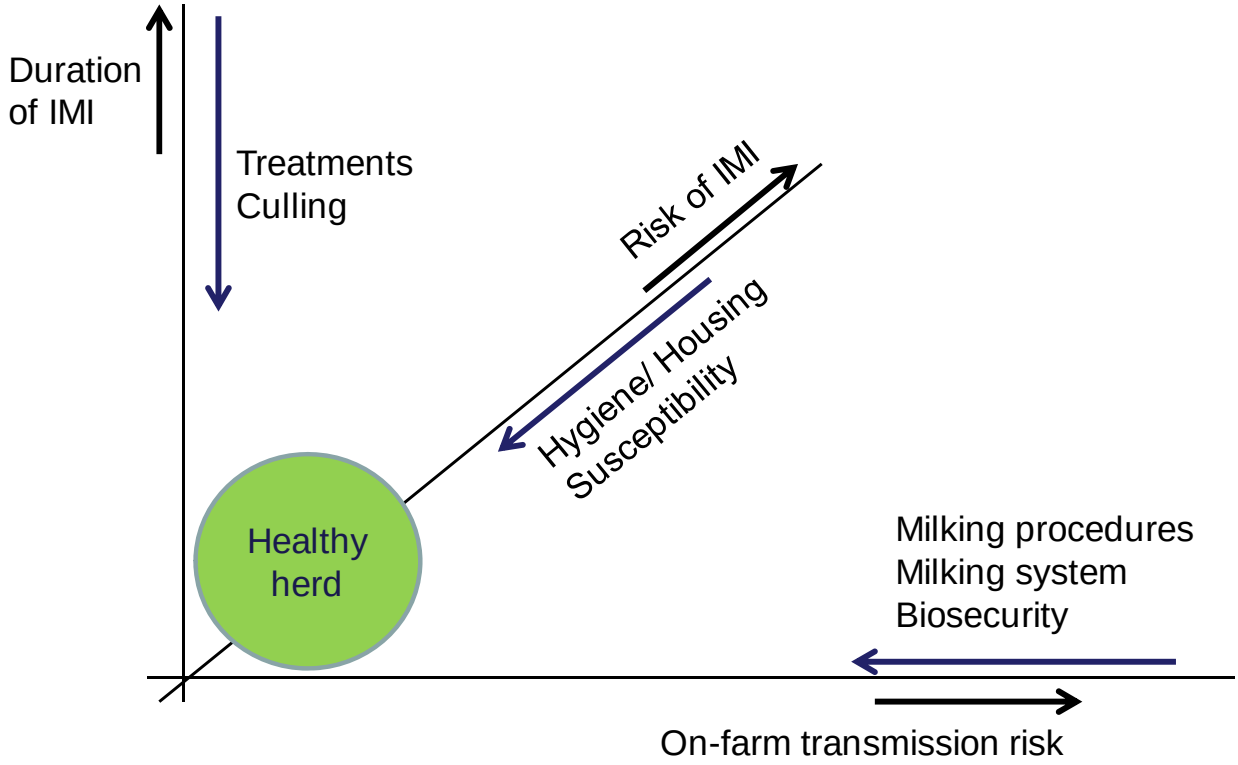


Figure 5. Least square means of bacteriological cure, clinical cure and clinical improvement by bacterial species and treatment arm. Cows with non-severe clinical coliform mastitis were randomized to obtain a 5-day ceftiofur treatment or remained untreated as a control group. Data from Schukken et al. 2011.

Figure 6. Components of infection biology and management practices that make up a herd that has a healthy udder health status.



Improving Mastitis Treatments by Targeted Antimicrobial Therapy

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Introduction

Control of mastitis caused by Streptococcus agalactiae and Staphylococcus aureus has resulted in reductions in bulk tank somatic cell count (SCC) but many herds continue to struggle with treatment of clinical mastitis caused by environmental pathogens. On many modern dairy farms, mastitis is caused by an increasingly diverse group of opportunistic pathogens (Figure 1). Common environmental mastitis pathogens include both Gram negative bacteria and Gram positive bacteria (Figure 1). The presentation of the symptoms and duration of infection is associated with the degree of host adaptation of the pathogen. Some environmental pathogens (such as most E. coli), are truly opportunistic and the immune response successfully eliminates them after a brief period of mild clinical disease. Other environmental pathogens (such as Streptococci spp.) have become more host adapted and may present as mild clinical cases that erroneously appear to resolve when the case has actually returned to a subclinical state. Both of these scenarios make it very difficult to determine success of mastitis treatments. While farmers often remember the most severe cases of mastitis, research demonstrates that the majority of clinical mastitis cases are mild to moderate in severity. The purpose of this presentation is to review research based principles that can help improve treatment of clinical mastitis.

Figure 1. Results of milk samples submitted from 793 cases of clinical mastitis occurring on 51 large Wisconsin dairy farms in 2010.[1]

DETERMINING RELEVANT OUTCOMES OF MASTITIS THERAPY

It is often difficult to determine if mastitis treatments are successful because there is no standard outcome that is used to evaluate outcomes. The detection of mastitis is based on recognition of the immune response that is a result of the infection. Thus, interpretation of treatment outcomes can be confusing because, clinical signs will normally resolve within 4-6 days, regardless of treatment. This is expected, as the response of immunologically competent cows will often successfully reduce the number of bacteria infecting the gland. However, disappearance of clinical signs does not always indicate that the infection has been successfully eliminated. As the immune response lessens, the milk may return to normal appearance, however many of these cases may have simply regressed to a subclinical state and maintain increased SCC. This occurrence is especially true for Gram positive pathogens.

The ability to achieve a bacteriological cure depends on the pathogen, case severity, variation in immune response among cows, efficacy of the treatment protocol (when needed) and the promptness of initiating treatment.[2] Even in the absence of mastitis caused by Staph aureus, bacteriological cures are almost always greater for Gram negative as compared to Gram positive pathogens (Figure 2).[1]

Figure 2. Treatments outcomes based on comparison of microbiological results of Milk samples collected at detection of clinical case and follow-up samples collected 3 weeks later.[1]

In one study, bacteriological cure was 7 times more likely for first cases of mastitis as compared to

recurrent cases.[3] Definition and interpretation of bacteriological cure also depends on laboratory procedures as differences in laboratory protocols can influence the probability of recovering bacteria from milk samples. Issues such as the frequency of sampling, the volume of milk that is inoculated, the time period after therapy until sampling and time between collection of consecutive samples all contribute to the wide variation in bacteriological cure rates noted in the literature.[4]

On a practical basis, farmers often assess clinical efficacy based on the appearance of the milk or other indicators such as recurrence of another clinical case, reduction in SCC, return of milk yield to normal, retention of the cow within the herd and number of days milk is discarded (because of abnormal appearance or the presence of antibiotic residues). Recurrence of another case of clinical mastitis is one of the least desirable outcomes after treatment and may be more likely for cases that occur early in lactation as compared to cases that occur later (Figure 3)[3]. This may indicate the need for more aggressive treatment protocols (for example, longer duration therapy) for cows experiencing mastitis in early lactation as compared to treatments for cases that occur later.

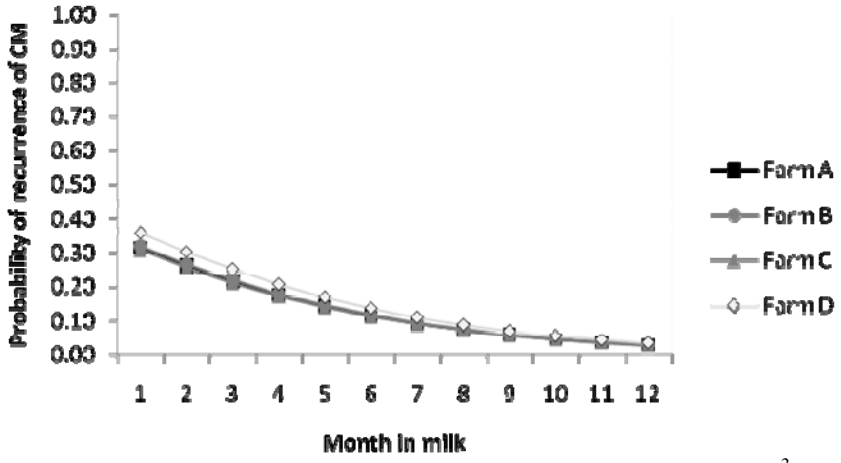

Figure 3. Recurrence of clinical mastitis (CM) by stage of lactation[3]

Somatic cell reduction below 200,000 cells /mL is another desired outcome but occurs slowly and this outcome is highly influenced by pathogen. Of cases caused by Gram-negative pathogens or no growth 63% resulted in somatic cell reductions to less than 200,000 cell/mL within 21-55 days after treatment in contrast to only 44% of cases caused by Gram-positive bacteria.[3] While long-term reductions in SCC should occur after successful therapy, short-term changes in SCC should not be used to determine when to stop therapy nor to determine if therapy has been effective. Likewise, the use of cowside tests like the California Mastitis Test should not be used to determine when to stop treatment.

Cow Factors Influencing Treatment Outcomes.

Host factors are well known to influence the probability of success responses to mastitis infections.[5] Older cattle have a greater risk of both subclinical and clinical mastitis and several studies have indicated that older cattle have poorer responses to treatment as compared to younger cattle.6 Deluyker et al., used a rigorous definition of clinical cure (normal milk by 5 d and no relapse within 3 weeks post-treatment) and reported a reduction in combined "clinical & bacteriological cure rates" from 39% (lactation 1) to 26-30% for older cattle.[7] Other researcher have reported that bacteriological cure after mastitis therapy were less for older cows.[8-11] Age has also been associated with reduced clinical responses to

therapy. Hektoen et al., measured responses to treatment by comparing scores for both acute and chronic symptoms obtained before treatment and at various periods post-treatment.[12] While parity was not associated with differences in acute symptoms, the reduction in chronic symptoms (changes in the milk, gland or inflammatory response) were markedly greater in first lactation as compared to older cattle. The effect of parity should be considered by practitioners before initiating mastitis treatments. For example, when IMM compounds are approved for extended duration therapy, veterinarians may want to consider using use longer duration of treatment for cases occurring in older cows. Likewise, older cows (>3 lactation) may not be good candidates for withholding treatment if that option is used for managing some cases of mastitis on particular farms.

Differences Among Pathogens

It is well known that mastitis is caused by a diverse group of bacteria and the probability of cure is highly influenced by the characteristics of the pathogen. The pathogenesis, virulence and prognosis of IMI is influenced by important characteristics that vary among pathogens. Depending on specific virulence factors, organisms infect different locations in the mammary gland, have differing abilities to cause systemic symptoms, vary in the expected duration of subclinical phases of infection and differ in the expected rate of spontaneous bacteriological cure. Understanding these differences is fundamental to development of effective control programs. For example, expectations for spontaneous bacteriological cure of subclinical and clinical mastitis caused by Staph aureus are essentially zero[13] while the expectation for spontaneous cure of E coli is quite high.[14] While a few cases may result in spontaneous cure, therapeutic cure rates for several mastitis pathogens (yeasts, pseudomonas, mycoplasma, prototheca etc.) are essentially zero, regardless of treatment. Even among Gram-positive pathogens, outcomes vary. The following typical differences among pathogens in bacteriological cure after treatment have been noted: Strep uberis (89%, n = 488 cases); Strep dysgalactiae (69%, n = 32 cases), Staph aureus (33%, n = 40 cases), and CNS (85%, n = 71).[9] On farms that have controlled contagious mastitis, approximately 25-40% of clinical cases are microbiologically negative before treatment. Clinical and spontaneous cure rates for these "no-growth" samples are often very high with or without treatment.[15,16]

Most cases of clinical mastitis caused by E coli are detected well after the immune response of the cow has been initiated and the immune response is usually successful in eliminating IMI caused by E coli. However, the duration of IMI caused by other coliforms (such as Klebsiella or Enterobacter) is much longer. After coliform bacteria infect the mammary gland, they multiply rapidly but most do not adhere to or invade the epithelial cells.[5] If the cow's immune response is rapid and efficient, infection will be quickly eliminated and there will be little long-term impact on cow health or productivity. The outcome of clinical mastitis caused by coliform bacteria depends on the severity of the case, which is usually dependent on the balance between the dose (relative degree of exposure to bacteria) and the ability of the cow to response immunologically. Severe cases of mastitis occur most frequently in the periparturient period and early lactation and are primarily associated with characteristics of the cow that influence her ability to respond to the infection.[5,17-19] When influx of neutrophils is delayed or phagocytosis or intracellular killing mechanisms of neutrophils impaired, bacterial multiplication continues, resulting in greater concentrations of inflammatory mediators and more severe clinical disease

In contrast, mastitis caused by environmental Streptococci typically respond well to IMM antimicrobial therapy but have a low spontaneous cure rate and high rate of recurrence when antimicrobials are not administered.[16] These differences among pathogen demonstrate

that identification of pathogen considerably improves mastitis treatment protocols. With current laboratory methods, it is not feasible for all farms to achieve a microbiological diagnosis before beginning therapy but guiding treatment by use of on-farm culture systems (OFC) has been shown to be economically beneficial.[20,21] The use of OFC to direct treatment of clinical mastitis gives farmers the opportunity to make better treatment decisions and reduce costs associated with milk discard and treatment of microbiologically negative cases. A positively controlled clinical trial evaluating OFC demonstrated that there were no significant differences in either long-term or short-term outcomes for cases of mastitis that received treatment based on results of OFC as compared to cases treated immediately without regard to diagnosis.[20,21] In this study, antimicrobials were not administered to cases that were culture negative or Gram negative thus the use of intramammary antimicrobials was reduced by approximately 50% as compared to cases which were treated without prior diagnosis. Most smaller herds cannot adopt OFC and an alternative is to encourage veterinary clinics to offer in-veterinary clinic culturing (IVCC). In these instances, farmers initiate treatment immediately but may modify treatment duration or drug after receiving a preliminary microbiological diagnosis within 24 hours. Development and oversight of a culture program (either OFC or IVCC) is an ideal way for veterinarians to increase involvement in mastitis control programs. The use of veterinary technicians to supervise these programs may also increase veterinary involvement and oversight of mastitis treatments. Veterinary technicians can visit farms to restock supplies, train farm personnel and provide oversight and quality control.

Duration of Therapy

In general, duration of antibiotic treatment should be kept as short as possible to minimize the economic losses associated with milk discard while maximizing the probability of achieving bacteriological cure. The appropriate duration of antibiotic treatment for clinical mastitis has not been well-defined and varies depending on the causative pathogen. There is considerable evidence that extended administration of antibiotics increases cure rates for pathogens that have the ability to invade secretory tissue (Staph aureus and some environmental Streps). For example, bacteriological cure for subclinical mastitis caused by Staph aureus treated with IMM ceftiofur were 0 % (no treatment), 7% (2 days), 17% (5 days) and 36% (8 days).[13] Cure rates reported for clinical mastitis caused by β-lactamase negative Staph aureus were significantly greater when extended duration therapy was used (50%) versus administration of 3 treatments over 36 hours (38%).[8] Likewise, bacteriological cure rates for experimentally induced Strep uberis infections increased from 58% (2-d treatment) to 69-80% for treatments of 5 or 8 days.[22] Therefore, for mastitis caused by potentially invasive pathogens, the duration of therapy should be 5 to 8 days. Research to support use of extended duration therapy to treat pathogens that infect superficial tissues (for example coagulase negative staphylococci or most E. coli) has not been published and the use of extended duration therapy to treat these pathogens significantly increases costs without improving treatment outcomes.[23] When extended therapy is considered, veterinarians should assess the ability of the herd personnel to perform aseptic infusions as extended intramammary treatment is associated with an increased risk of infection from opportunistic pathogens, and herds with poor infusion techniques are not good candidates for multiple doses of intramammary tubes.

Conclusion

Veterinarians should be involved in developing and implementing mastitis treatment protocols and should work with farm personnel and other professionals to actively monitor outcomes of treatments that farm personnel administer. Research evidence is available to help guide mastitis treatment decisions and to better select animals that will benefit from

specific treatments. There is sufficient research evidence to help develop mastitis treatment protocols that vary depending on animal characteristics and the history of subclinical disease. The use of OFC or IVCC is an ideal way for veterinarians to become more involved in helping farmers make rational decisions about antimicrobial therapy used for treatment of mastitis.

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Continuity of business during a FMD outbreak: Secure milk supply project

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Introduction

In the event Foot and Mouth Disease (FMD) is diagnosed in the United States, a national animal health emergency will be declared and livestock and allied industries will feel the immediate impact of animal and animal product quarantine and movement restrictions. Quarantine, managed movement and mandatory biosecurity protocols are designed to contain and control the disease and minimize virus spread. However in the dairy industry, the just-in-time supply practices of milk movement in the U.S. could be significantly impacted by managed movement and the need for additional biosecurity at farms and processing facilities. This could lead to a disruption of the provision of milk and milk products to consumers, along with the potential for significant milk disposal and animal welfare issues on dairies.

Most dairy operations and processing plants do not have the capacity to store milk for more than 48 hours; some have less than 24 hours storage capacity. Hence, preplanning for safe, timely, risk-based, permitted movement of animals and animal products will be critical to maintaining the business continuity of the dairy industry while controlling and containing the outbreak.

Goals

The goals of the national Secure Milk Supply (SMS) Plan are to maintain business continuity for dairy producers and processors during an FMD outbreak, to minimize disease spread, and to assure a continuous supply of milk and milk products to consumers. The specific aims of the SMS Plan are to 1) Engage stakeholders in the planning process for an FMD response, 2) Develop and socialize tools and guidance documents that support business continuity within the dairy industry, and 3) Ensure that producers, processors, federal and state agency personnel agree the proposed guidelines are feasible, implementable, and effectively enable critical movements of animals and animal products with minimal risk of further FMD spread during an outbreak response.

Focus

Initial the project is focusing on the movement of raw milk from farm to commercial processing, and the development of resources and tools to facilitate this type of movement while minimizing the risk of FMD spread. The development of these tools and resources is being done through Public-Private Partnerships that involve stakeholders from the dairy industry, State and Federal agencies, and academia working through working groups. Current components being developed include:

Biosecurity Performance Standards

National biosecurity performance standards for dairy premises, milk haulers, and processing plants have been developed for implementation during an FMD outbreak. Compliance with these performance standards is intended to significantly reduce the chance of spreading FMD virus while facilitating the ability to permit raw milk movement from dairy premises (not known to be infected with FMD) in a Control Area to processing where it can be pasteurized for commercial consumption. The dairy industry and Animal Health Officials in each state are encouraged to develop pre-event, standard operating procedures that meet the national performance standards while accounting for the local climate and industry practices.

Decision Support Tools

As an aid to timely decision making at the onset of an FMD outbreak, a proposed framework for classification of an outbreak response based on the phase (time course of the event) and type (scale or magnitude of the event) has been developed to facilitate response planning. In addition a set of recommendations pertaining to raw milk handling and processing has been drafted for pre-event review, discussion, and ideally agreement with incorporation into local/regional/national response plans.

Active Observational Surveillance (AOS) Training Materials

AOS as part of the SMS Plan is "an active process for the detection of foot-and-mouth disease on dairy premises, utilizing trained observers (herd managers or workers) who are routinely monitoring animals on a daily basis for abnormal or increased occurrence of clinical signs compatible with FMD, or changes in food or water consumption, or milk production. AOS does not replace the need for periodic inspection of the herd by animal health officials; it increases the likelihood of early detection of FMD by trained Herd Health Monitors.

Proactive Risk Assessment

Risk assessments support managed movement and permitting of animals and animal products during disease outbreaks. As part of the SMS Plan, proactive risk assessments are being conducted to evaluate the risk that the transport of raw milk from an FMD infected, but undetected, dairy farm to further processing poses to the spread of FMD. The initial risk assessments consider current Grade A milk production practices as well as proposed mitigations developed through the SMS effort and the risk of virus spread through identified pathways onto or off of a farm via the transport of raw milk to further processing. The results of the proactive risk assessments will help inform movement and permitting decisions in the event of an outbreak.

Summary

The national SMS Plan has made significant strides in the development of a framework and support tools to facilitate decision making and timely permitting for raw milk movement during an FMD outbreak response. However, these tools need to be tailored to specific states and regions, discussed and agreed upon by those responsible for decision making at that level, and then incorporated into state and regional FMD response plans.

Raw milk movement is just one of a number of critical movements needed for a dairy to remain in business and be economically viable. Future planning efforts should focus on other priority movement areas, such as contingency planning for dairies which utilize off-site calf rearing.

If FMD is diagnosed in the U.S., it will be a major animal health emergency and severely impact the daily activities and economic viability of all livestock sectors of the U.S. economy. With enhanced contingency planning and clear communications between industry and government prior to an outbreak, we can ensure significant improvements in the national resiliency of U.S. livestock industries to transboundary animal diseases and hence enhance the security of U.S. livestock and food production systems.

For more information, visit the Secure Milk Supply website at www.securemilksupply.org

Impact of Emergency Vaccination in a Foot-and-Mouth Disease outbreak in Minnesota, USA

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Objective: The main goal of USDA and MN FMD response plans is disease eradication. The objective of this study was to evaluate emergency vaccination control strategies for a simulated FMD outbreak in Minnesota.

Methods: The North American Animal Disease Spread Model (NAADSM) was used to develop and compare scenarios that varied based on whether vaccination was utilized, the time to deliver vaccine, the capacity to administer the vaccine and the time to develop immunity. Output data analyzed included the mean number of infected herds and animals, mean duration of active FMD disease, mean duration of outbreak, and mean number of herds and animals vaccinated. Important elements included movement restrictions, depopulation, and surveillance, using MN-specific direct and indirect contacts between herds, and airborne spread. Simulated FMD outbreaks (1,000 model iterations) began in and were limited to MN. Vaccine related variables explored included time to deliver vaccine (7, 14 and 21 days), time to develop immunity from vaccine (4 and 7 days), and number of herds vaccinated per day (two levels: 50 (assumption with federal/state veterinarian applied vaccination) and 1,500 (assumption with industry vaccinators under the supervision of accredited veterinarians) herds per day).

Results: Our study described the implications of emergency vaccination and compared the epidemiological results of FMD in MN with and without emergency vaccination. Results suggested that vaccination had important implications in a MN outbreak and was associated with large differences in disease and outbreak duration and number of animals/herds infected. These results were more striking for scenarios in which disease begins in a dairy. Assuming a dairy herd was initially infected, the mean number of animals infected ranged from 30,000 to 88,000 with 50 herds per day vaccinated and varying delivery and immunity time. However, when vaccination capacity was increased to 1,500 herds per day and other conditions held constant, the mean number of animals infected was consistently below 20,000. Variability around means also decreased with vaccination.

Conclusions: Models that began in a Dairy Index herd showed greater response to vaccination effect across all measured outputs. The application of a large scale, rapidly administered, emergency vaccination program (1500 herds vaccinated per day) greatly diminished the duration and severity of an FMD outbreak, assuming a Dairy Index Herd.

Update on Milk Residues

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Milk residues continue to be a hot topic among dairy industry personnel and within veterinary circles. As the industry and government await the results of FDA's sampling surveillance project, questions continue to circulate regarding the impacts of the project, future actions and changes in residue testing and the regulatory requirements. Almost certainly, the results of a small sampling surveillance project designed to address a specific risk factor on dairy farms, tissue residue violations, will have a much wider impact when the results are released.

Traditionally, drug residue testing on milk has been limited to the beta lactam testing requirements explicitly stated in the Pasteurized Milk Ordinance (2011 PMO, Appendix N, p. 342):

"Industry shall screen all bulk milk pickup tankers, regardless of final use, for Beta lactam drug residues."

In Minnesota, the results of this testing program have shown steady reductions in the number of violations obtained each year (Table 1).

Table 1. MN Drug Residue Summary 2007-2012

Year	2007	2008	2009	2010	2011	2012
Number of Residues	133	97	81	92	89	56
Average Number of Farms	5067	4864	4689	4511	4333	4090
Percent of Farms with Violations	2.62%	1.99%	1.73%	2.04%	2.05%	1.37%

The current testing regimen has been consistent for many years and is a very predictable, very structured process which allows dairy producers and industry to understand exactly what is being tested for, when tests are occurring and allows them to put standard procedures in place to avoid residues.

The potential addition of testing requirements is a very real possibility. This requirement may come from additional government regulations; however, it is much more likely that the first testing programs outside of the stated regulatory requirements will be performed at the initiative of the industry in response to the expectations of other countries or customers. In fact, some of this testing is

already beginning to take place. The additional testing creates an environment which is much less predictable for the dairy producer, processor and those veterinarians serving the dairy industry.

Additional testing protocols are not as well defined as those that exist to meet the prescribed regulatory requirements. Testing may be done for a wider variety of drugs, on a longer timeline, and using tests which have not been officially approved by the FDA. All of these factors make it much more difficult for producers to predict when testing is occurring, what drugs are being tested for, the implications of a positive result and how to prevent a violation. In general, these factors create an environment in which producers and veterinarians must be extremely vigilant about how they are using drugs on their farm, following the proper withdrawal times and ensuring they have the proper controls in place to prevent a residue for a drug, regardless of which drugs they are using. Judicious drug use and drug treatment protocols on farms will continue to be critical to ensuring a safe milk supply and preventing drug residues.

Johne's disease: A zoonosis?

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Most cattle veterinarians are familiar with Johne's disease (JD). The disease is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and has been described worldwide. The infection progresses slowly so that most infected cows do not show clinical signs of this granulomatous enteritis, chronic diarrhea and wasting, until they are 2-4 years of age. The predominant transmission route is fecal-oral and infected cows shed the bacterium through manure, milk, and colostrum. It is estimated that 70% of dairy herds in the United States are infected with a within-herd prevalence of 3-5% test-positive animals[1]. Johne's disease has an economic impact on infected farms through premature culls and reduced milk production.

While Johne's disease is primarily a ruminant disease (i.e., cattle, small ruminants, camelids, cervids), clinical disease after natural exposure has also been described in a donkey[2] and MAP was isolated from other monogastric animals such as bears and cats[3]. A human disease that is often compared to JD is Crohn's disease (CD) as the gross pathology of both diseases looks very similar. It has been speculated whether MAP is a zoonosis and could be a potential cause of CD. Both is unclear at this point. However, the hypothesis cannot be completely dismissed with the current knowledge as MAP belongs to the genus *Mycobacterium*, which includes severe human diseases such as M. tuberculosis (human tuberculosis), M. bovis (bovine tuberculosis, zoonotic) and M. leprae (leprosy). Therefore, the dairy industry is concerned that consumers could perceive a causal relationship between MAP and CD - whether a causality is actually scientifically proven or not. The biggest problem is that MAP can survive for long times in the environment and is furthermore very heat resistant. Pasteurization does only reduce its numbers[4] and viable MAP has been isolated from environmental[5] and drinking water[6], infant formula[7], pasteurized shelf milk[4] and beef[8]. Only thorough cooking of beef (to well done) will kill viable MAP in meat[9].

Crohn's disease and JD are both chronic diseases and the onset of clinical disease is most commonly after puberty, i.e., at 15-30 years and 50-70 years of age in humans and 2 years in cattle. However, unlike JD, CD can affect any portion of the gastrointestinal tract from mouth to anus and even inflammation of skin, joints and eyes are common. Most patients with CD suffer from severe abdominal cramps, nausea, chronic diarrhea, vomiting, fever, ulcers in mouth or at anus. The disease is a transmural inflammation of the intestine. It is generally recognized that

there are 5 different types of CD, depending on the affected portion of the intestinal tract, and that Crohn's disease should therefore be more appropriately used only as an umbrella term for "Crohn's diseases"[10]. Ulcers, fistulas or strictures of the gut are common, which require a surgical resection of parts of the intestine in roughly 75% of Crohn's patients. The diagnosis of CD is based on a battery of tests (blood tests for anemia and inflammation, liver function tests, stool analysis, endoscopy and gut biopsy, x-rays with barium contrast) and other exclusion criteria. A differential diagnosis of CD is ulcerative colitis (UC) that also belongs to the inflammatory bowel disease (IBD). In 10% of patients the features are undistinguishable between UC and CD and no clear diagnosis can be made[10]. The prevalence of CD patients has been estimated at 1 in 694 people in the United States has the disease. Manitoba, Canada, has the highest CD prevalence with 1 in 428 people. However, over the recent years the incidence of IBD has increased significantly[11].

The etiology of CD is still not fully understood despite the fact that CD was already first described in 1904. The different forms of CD and the fairly unspecific diagnosis might contribute to this dilemma. Although CD was first thought to be an autoimmune disease[12], now it is generally accepted that CD is a multifactorial disease. Factors that are associated with the disease include genetic susceptibility, westernization of the lifestyle, dietary changes towards fast-food, changes in alimentary microbiota, hygiene, various possible pathogens and treatment with antibiotics in the first year of life[11,12].

The genetic component of CD has been established based on the observation that certain ethnicities have a higher incidence of CD than others, that the odds to get CD are higher if a family member has CD and specific gene defects were discovered in some patients with CD[13]. For example, defects of the NOD2 receptor have been described by 3-43% of CD patients [14] - as well as in cows with JD[15]. However, the genetic susceptibility alone does not produce IBD and it also cannot explain the relatively quickly rising incidence of IBD over the past decades as the genetic pool was relatively stable[16]. Therefore, the role of various "environmental" factors needs to be discussed[11,13]. In particular the role of a changed gut microbiota in the disease etiology is more and more under investigation. Although, it is currently still unclear if general changes in the gut microflora or specific pathogens are the major contributor to CD pathogenesis[13].

Factors that were identified to be associated with an increased incidence of IBD and CD in a population include increased stress levels, smoking, obesity, westernization of society and changes in diet to include more fast-food, dairy products, meat and fatty acids[11,16]. Furthermore, studies have found that children treated with antibiotics in their first year of life were 5.3 times more likely to develop CD[17]. As the microflora well as the immune system of the GI tract develop in the first 5 years of life in humans, both will likely been altered by antibiotics in early childhood[11,13]. Contrary, poor domestic hygiene, parasitism, and overcrowding reduce the risk of CD. In particular the latter supports the hypothesis regarding a change in the general gut

microbiom and contradicts theories of a specific pathogen as those circumstances would support the involvement of a transmissible factor[13]. Nevertheless several pathogens are being discussed in association with CD. They include MAP, *Yersinia ssp., Mycobacterium tuberculosis, Helicobacter hepaticus,* Adherent-Invasive *Escherichia coli (AIEC), Listeria* ssp., *Entamoeba histolytica*, *Campylobacter,* an early measles (Paramyxovirus) infection and more[12,13]. The diversity of pathogens under investigation shows that at this point no single pathogen has been identified the cause of CD in humans and many questions remain. In the case of MAP, there are studies that found higher presence of MAP in CD patients. For example, Bull et al.[18] found MAP in up to 92% of CD patients compared to 23% of controls. However, other studies were unable to find MAP in Crohn's patients or to determine an association between MAP with CD[19]. Therefore, one might speculate that MAP is a part of the causal mix in some cases of CD. However, unless more specific tests for the diagnosis of CD and its types are developed this is difficult to prove. Crohn's disease is not a homogeneous disease but rather an umbrella-like term of several multifactorial diseases and trying to explain MAP as cause for all CD cases is comparable to saying that all calfhood diarrhea cases are attributable to e.g. *Salmonella*.

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Temporal and spatial patterns of cattle imports to Minnesota for the years 2009 and 2011: Identifying cattle farms and zones for risk-based surveillance

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In this study we propose to develop a risk-based surveillance system for MN cattle herds by creating a risk profile of each import cattle movement to the state of MN. We hypothesize that by doing so, we will identify high risk herds and zones for BTb introduction into the state of MN, which can direct resource allocation for the state animal health agency.

Data on import interstate cattle movements was obtained from the Minnesota Board of Animal Health (MNBAH) for the years 2009 and 2011. Descriptive analysis was performed for 2009 and 2011. Movement data was summarized at the premise and county level and for both levels the distribution of cattle moved and number of movements was evaluated. In order to develop a strategy for a targeted surveillance system at the herd level for BTb in MN, each import movement was risk profiled based on known risk factors. The data was analyzed by fitting a linear regression model at the county level, to determine which variables might be affecting higher number of movements at the regional level.

In each year, about 1500 herds had import movements to MN. Most of the cattle imported were in the categories of beef and feeder cattle, mostly originated from bordering US states. The peak season for incoming cattle and import movements was the fall season for both years. The risk model identified four risk groups with about 500 (~2% of total cattle farms in MN) cattle premises in the Very High and High risk group for each year. The southeast and southwest zones of the state had the highest density of cattle premises with movements and also cattle premises in the higher risk groups.

In this abstract an approach for risk-based surveillance for BTb is presented using available data from the MNBAH, which outlines a method that can be used in the US for BTb and other disease scenarios.

Effect of Using the Perfect Udder® System to Heat-Treat Colostrum on Passive Transfer of IgG in Neonatal Jersey Calves

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Heat-treating colostrum using batch pasteurizers has been proven effective to reduce bacterial contamination while protecting immunoglobulins, and enhancing passive transfer and health in calves. The Perfect Udder® bag (Dairy Tech Inc., Windsor, CO) is designed to heat-treat colostrum (then store and feed) one gallon at a time. However, this system requires validation. The study objective was to describe the effect on passive transfer of IgG in neonatal calves when using the Perfect Udder® heat-treatment system as compared to a negative control (fresh refrigerated or fresh frozen colostrum) and a positive control (batch heat-treated colostrum).

The study was conducted in summer, 2012, on a large commercial Jersey farm in Minnesota. First milking colostrum was pooled each day to achieve a unique batch. The batch was then divided four ways with 3.8 L allocated to each treatment group: 1. Heat-treat in Perfect Udder® bag at 60°C for 60 minutes (PU); 2. Batch heat-treat at 60°C for 60 minutes and store in Perfect Udder® bag (BT; positive control); 3. Fresh frozen in Perfect Udder® bag (FF; negative control); 4. Fresh refrigerated at 4°C in Perfect Udder® bag (FR; negative control). Colostrum from all treatments was sampled for IgG concentration and bacterial culture testing immediately after assembly, post processing, and post thawing/prior to feeding. Newborn calves were removed from the maternity pen within 20-30 minutes of birth and before suckling. The calf was weighed and an 8 mL blood sample was collected from the calf's jugular vein prior to feeding. Singleton calves were randomly assigned to one of four treatment groups (PU, n=28); (BT, n=28); (FF, n=29); (FR, n=27). At 24 hr of age, a second 8 mL venous 'postfeeding' blood sample was collected. Paired serum samples collected at 0 and 24 hrs were analyzed for IgG (mg/ml) using RID analysis.

Mean dystocia score, calf weight, and quality of colostrum fed (g/L IgG) were not different among colostrum treatment groups. The mean age at feeding in minutes was shorter for calves fed FR colostrum (69 min), as compared to PU (79 min), BT (83 min) and FF (88 min) groups. However, age at feeding was not associated with IgG absorption in this study. Final analysis of variance models showed a significant effect of treatment on IgG absorption when comparing fresh (FF or FR) versus heat-treated (PU or BT) treatment groups, but no difference between FF and FR treatments and no difference between PU and BT treatments. The mean apparent efficiency of absorption of IgG (%) was 37%, 37%, 32%, and 32% for the PU, BT, FF and FR groups, respectively. The mean final serum IgG value at 24 hrs was 41.1, 40.0, 35.2, and 35.7 mg/ml for PU, BT, FF, and FR groups, respectively. Secondary analysis indicated that enhanced efficiency of absorption of IgG and final serum IgG concentrations for calves in the PU and BT groups was likely attributed to the fact that lower bacteria counts in heat-treated colostrum, and particularly lower coliform counts, are associated with enhanced IgG absorption. Pre-feeding total plate count (log TPC, cfu/ml) was significantly different for all 4 treatments, with mean

values of 4.23, 3.63, 5.68, and 6.53 for PU, BT, FF and FR groups, respectively. Total coliform count (log TCC, cfu/ml) was also significantly different for all 4 treatments, with mean values of 0.45, 1.08, 3.82, and 4.80 for PU, BT, FF and FR groups, respectively.

Calves fed colostrum processed using the Perfect Udder® heat-treatment system had improved efficiency of IgG absorption and final serum IgG concentrations as compared to fresh colostrum groups. However, efficiency of IgG absorption and final serum IgG concentrations were not different for calves fed colostrum heat-treated using the Perfect Udder® system as compared to batch heat-treated colostrum. The Perfect Udder® colostrum heat-treatment system can be useful to dairies desiring to heat-treat 1 gallon aliquots of colostrum at a time.

Supplemental fat for dairy calves fed accelerated milk replacer during mild cold stress[1]

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INTRODUCTION

One of the most commonly experienced stressors in livestock is caused by fluctuations in environmental temperature that extend beyond the thermonuetral zone for the animal. The reported lower critical temperature (**LCT**) for a newborn dairy calf ranges from 13 °C (Curtis, 1974) to 8°C (Young, 1981). Limited data are available evaluating impact of supplemental fat for nursery calves fed accelerated milk replacer during cold stress. Questions remain regarding when to feed and amount of supplemental fat to optimize calf nutrient intake for calf growth and health. Numerous studies have investigated effects of varying the fatty acid profile of calf milk replacer; however, we are unaware of studies designed to specifically target amount of supplemental fat fed during the early nursery phase (Jenkins et al., 1985; Piot et al., 1999; Gaulet et al., 2000; Hill et al., 2007; Mills et al., 2010). Increasing rates of milk replacer feeding have been shown to reduce starter intake and delay rumen development (Bar-Peled et al., 1997; Jasper and Weary, 2002; Terré et al., 2007). Supplemental fat may be optimally positioned early in the nursery phase (first three wk) when nutrient intake from starter is expected to be low. Khan et al. (2007) found that providing calves a large amount of milk early in life and then reducing milk intake before weaning (step-down method) caused a surge in solid feed consumption. Removing supplemental fat from milk replacer could be considered as an alternative approach to inducing a similar step-down effect on starter intake without altering the amount of milk replacer fed. Cold stressed calves may benefit from supplemental fat by sparing glucose and amino acids which are used for thermoregulation when calves are managed in conditions below the thermal neutral zone. Composition of maternal transition milk indicates that calves are naturally programmed to consume high fat milk during the early days of life. Increasing amount of fat fed to calves in the beginning of the nursery phase may replicate added energy from milk fat which is typically at an elevated concentration in early lactation. Supplemental fat may spare amino acids (alanine) by reducing amount of amino acids catabolized for energy resulting in a greater proportion of dietary energy available for lean growth. In order to test this hypothesis milk replacer must be fed that provides sufficient amounts of protein to meet the amino acid requirements of nursery calves for growth and maintenance (Bartlett et al., 2006; Donnelly and Hutton, 1976). Supplemental fat also spares energy that can be used for functions beyond growth including immune function. Reducing effects of cold stress on nursery calves may also reduce stress which has been shown to reduce efficiency of growth. Enhanced early nutrition capitalizes on growth performance early in life being driven more by increasing protein and lactose intake than by increasing fat intake (Tikofsky et al., 2001; Hill et al., 2008). Objectives of this experiment

were to determine if increasing fat intake during the first 21 d of the nursery phase alters growth rate, efficiency of growth, voluntary starter intake and nutrient intake in nursery calves fed accelerated MR and to determine if response in calf performance is altered by supplemental fat amount. We hypothesized that supplemental fat will decrease starter intake during the first mo of the nursery phase but energy available for growth will be greater resulting in increased calf growth.

MATERIALS AND METHODS

Animals

Experimental protocol was reviewed and approved by the University of Minnesota Institutional Animal Care and Use Committee. Eighty-one (n = 27 per treatment) Holstein and Holstein-cross calves born at the University of Minnesota Dairy Teaching and Research Center, St. Paul, MN were enrolled in the study. At birth, calves were removed from their dams, identified with a unique ear tag, birth body weight (**BBW**) was recorded, navels were dipped, and each calf remained in a heated indoor pen for the first 48 h after birth. Calf BBW averaged 41.8 ± 1.6 kg. Calves received approximately 1.9 L of colostrum at each of the first three feedings (within 2 h after birth and again approximately 12 and 24 h after the first feeding) and were trained to drink MR from buckets during the first 4 d of life. A blood sample was collected via jugular venipuncture into evacuated serum collection tubes (SST; Beckton Dickenson Vacutainer Systems, Franklin Lakes, NJ) 24 h after birth and centrifuged at 2,000×*g* for 20 min. Serum was separated and analyzed for total serum protein concentration using a refractometer (Reichert Rhino VET360, Reichert, Depew, NY). Serum total protein averaged 5.8 ± 0.1 mg/dL. Each calf was placed in a calf hutch (PolyDome, Litchfield, MN) bedded with wheat straw. Throughout the experiment the mean temperature was 1.7°C and the average low temperature was -3.9°C (Figure 1).

Assignments to Treatments and Feeding

Experimental design was a randomized block design with 3 treatments. Calves were assigned to treatment at birth and treatments were balanced by BBW, gender, breed, and serum total protein. Calves were fed one of three treatments; 1) Low fat (**LF**) (28:15 MR); 2) Medium fat (**MF**) (28:15 MR + 113 g/d commercial fat supplement (**FAT**) (60% fat); 3) High fat (**HF**); 28:15 MR + 227 g/d FAT. MF and HF calves received FAT 1 to 21 d.
All calves were fed LF 22 to 49 d. Calf milk replacer was fed at 1.4% of BBW 1 to 10 d and then 1.8% of BBW 11 to 42 d, and 0.9% of BBW 43 to 49 d and CMR was reconstituted to 13% solids. Calves were fed CMR twice d at 0630 and 1730 in hutches. Intake of CMR was similar ($P = 0.41$) among treatments and averaged 34.2, 32.6, and 33.5 kg/calf for LF, MF and HF respectively. Calves were weaned on day 49 and remained in hutches to day 56. Calves were fed a commercial texturized starter grain *ad libitum* (19.2% CP on a DM basis) (**Table 1**). Approximately 4-10 L of drinking water was provided daily. Starter refusals were recorded daily and refusals of MR were recorded at each feeding. Calf BBW averaged 42.0 ± 1.1 kg/d and were similar among treatments ($P = 0.41$). Calves were born over a 5-mo period (October through January) however; ambient temperature 5.3 ± 1.1°C at birth was similar ($P = 0.82$) across treatments. Quality of calf bedding was evaluated using nesting scores when calves were lying down in hutches (1 = calf legs are completely nestled into straw; 2 = calf legs are partially

nestled into straw; 3 = calf legs are resting on top of straw). Nesting scores were similar (P = 0.65) among treatments.

Nutrient composition of milk replacers and starter is listed by treatment in **Table 1**. Milk replacer was formulated to contain 28% CP and 15% fat on an as-fed basis and actual analyzed CP and fat on a DM basis was 30.9% and 15.7%, respectively. Supplemental fat contained 7.7% CP and 57.4% fat on a DM basis. Fat supplement contained 25.4% palmitic, 12.6% stearic and 42.2% oleic acid (**Table 2**). Starter was prepared to contain 18% (as-fed basis) CP and was 21.8% on a DM basis.

Body Growth and Health Monitoring

Calves were weighed at birth and weekly thereafter each Friday at 0900 h. Body length (**BL**), heart girth (**HG**) (dairy calf weigh tape, Fort Atkinson, Nasco, WI), hip width (**HW**), withers height (WH) and hip height (**HH)** (measuring stick, Fort Atkinson, Nasco, WI), were also measured. Calculations of average daily gain (**ADG**) of body weight (**BW**) and stature were made from these measurements. Calves were observed at least twice daily from 1 to 56 d of age for general health, including appearance (alertness), and appetite (ability to consume feed). Fecal scores (**FS**); 1 = normal, firm and well formed, 2 = semi-formed, pasty, 3 = loose, but stays on top of bedding, and 4 = watery, readily absorbed into bedding, were recorded daily from 1 to 56 d of age. Scours were defined as FS $\geq$ 3. Scours were treated with oral electrolytes (Bounce Back, Manna Pro, Chesterfield, MO) and continued CMR feeding.

RESULTS

Calf management

There was no treatment effect on nesting score, fecal score or weekly measures of rectal temperature (**Table 3**). Nesting score decreased (P < 0.001) over time as initial straw bedding was compacted with use. Consistently low fecal scores were observed during this study and were similar among treatments. Rectal temperature tended (P = 0.09) to be highest on week 3 with an average of 38.3°C.

Starter and nutrient intake

Starter intake was lower (P = 0.038) for MF vs. LF and HF (**Table 4; Figure 1**). As expected, starter intake increased (P < 0.001) with increasing age (Table 4). Starter intake on d 21, when the fat supplement was discontinued, was greater (P < 0.003), and on d 42 was greater (P = 0.009) for LF vs. MF and HF. Starter intake on d 49, when calves were weaned from CMR, was greater (P = 0.032) for LF vs. HF with starter intake by MF being intermediate. By d 56, when the study ended, starter intake per day was similar (P =0.646) across all treatments. Starter intake expressed as a percentage of body weight was affected by treatments similarly as was starter intake. Total DMI (starter intake + CMRI + FAT) from d 3-49 tended (P = 0.062) to be greater for LF vs. MF and HF. Total DMI from d 3-56 followed a similar trend. Feed efficiency (gain:feed) through d 49 was greater for MF and HF compared with LF, but gain:feed through 56 d was similar among treatments. Day of age in which calves were consuming at least 250, 500, 1000 and 2000 g/d was calculated to determine if there were differences not only in amount of starter intake but also age in which calves consumed these targeted amounts of starter. Calves fed LF ate the targeted 250 g/d of starter intake 5.5 and 6.4 d earlier than MF and HF, respectively. Mean separation in starter intake was observed as early as day 14 of the trial, but

interestingly by d 56 all calves were consuming a similar amount of starter grain. Peak starter intake was defined as the greatest amount of starter consumed per d during the 56 d nursery phase. Starter intake peaked at 3.5, 3.3, and 3.2 ± 0.1 kg/d for LF, MF and HF, and was similar ($P = 0.233$) among treatments. Calculated metabolizable energy intake from CMR was similar ($P = 0.775$) across treatments. As expected, ME_I increased with fat supplementation by 0.28 and 0.59 ± 0.06 Mcal/d for MF and HF during the first 21 d in which fat was added to CMR (**Figure 3**). Metabolizable energy intake was greater on wk 1, 2 and 3 for HF vs. LF and MF. On wk 6 LF ME_i was greater than MF and on wk 7 LF ME_i was greater was greater than LF. Despite higher starter intake by LF, supply of ME_I from calf starter was not different ($P = 0.221$) among treatments. Due to greater starter intake by LF and higher energy intake through fat supplementation by MF and HF, total ME_I through 56 d was similar ($P = 0.332$) among treatments.

Calculated intake of crude protein from CMR and starter grain from day 3 to 49 and day 3 to 56 was similar ($P = 0.323$) among treatments; however calculated fat intake was increased ($P < 0.001$) from LF to MF and MF to HF (**Table 5**). Intake of C16:0 and C18:1 from CMR was similar among treatments. Calculated intake of C16:0 from fat supplement was 0.36 and 0.73 kg and intake of C18:1 was 0.60 and 1.21 kg during the first 3 wk for MF and HF respectively. Total intake of both C16:0 and C18:1 from CMR and FAT was increased ($P < 0.001$) by fat supplementation HF > MF > LF. Post-weaning measures of apparent total tract DMd was similar across all treatments.

Growth and structural development

Calves fed MF and HF had greater ($P < 0.05$) BW gain during wk 1 and 2 than LF. Very few calves lost weight during week 1 (n = 6); however, of those losing weight during the first wk, 4 were LF and 1 calf each were fed MF and HF. Body weight gain and ADG through 21 d continued to be greater ($P < 0.009$) for MF and HF compared with LF (**Table 6**). Average daily gain during wk 1-3 tended (treatment × wk, $P = 0.077$) to be greater for MF and HF vs. LF (**Figure 2**). There were no differences in growth measures of HH, WH, BL, HG, or HW among treatments. Differences in total gain and ADG observed in calves at 21 d were no longer apparent by 42 d. By 56 d, there were no differences in total BW gain among treatments, however, LF had greater ($P < 0.029$) hip height and tended ($P = 0.053$) to have greater WH compared with MF and HF. Interestingly, there were no differences in rates of growth between MF and HF.

DISCUSSION

Supplementing fat during the early nursery phase provides additional nutrients that more closely mimic fat concentration found in transition milk of the dam. Calculated as-fed protein to fat ratio for treatments employed in this study were 28:15, 26:20, and 24:25 for LF, MF, and HF respectively. The HF treatment most closely represents the protein:fat typically found in cow's milk. Although amount of fat supplied by HF is similar to that from cow's milk, fatty acid (FA) profile is considerably different lacking short (< 6 carbon) and medium chain (6 to 12 carbon) FA found in milk fat.

Starter and nutrient intake and feed efficiency

Lower starter intake by calves fed FAT through d 49 is not surprising as these calves likely experienced greater satiety with chemical and physical factors. Calves fed an enhanced-growth feeding program usually have lower starter intakes during the preweaning period compared with conventionally-fed calves. Calves consuming appreciable amounts of starter at a younger age may have advantages in gastrointestinal tract maturation, greater capacity for nutrient absorption, and increased gut integrity (Hill et al., 2010; Sweeney et al., 2010). An alternative view is a greater supply of energy and nutrients from higher milk intake could contribute directly to development of rumen papillae through metabolic axis (Shen et al., 2004). Interestingly, changes in endocrine factors (insulin and IGF-1) in relation to higher milk intake may also promote development of ruminal epithelium (Gerrits et al., 1998; Shen et al., 2004). Post-weaning measures of total tract apparent DMd were similar across all treatments. We anticipated that greater rumen development associated with greater amounts of starter intake for LF would result in greater DMd, however, greater starter intake and likely increased rates of passage of digesta resulted in similar DMd. Apparent DM, OM, NDF, CP, and gross energy digestibility was greater in control fed compared with accelerated fed calves one wk after weaning (Terré et al., 2007). Greater feed efficiency for fat supplemented calves is not surprising given greater ME_I and the higher coefficient of digestibility for supplemental fat vs. starter grain. Perhaps supplemental fat also increased efficiency of growth by supplying greater amounts of energy during the first three wk and therefore reduced the impact of mild cold stress on nutrient used for growth. Effects of supplemental fat on day of age to consistently consume targeted amounts of 250, 500, 1000 and 2000 g/d starter grain is intriguing and warrants further investigation to determine impact of reducing day of age to consume benchmark amounts of starter.

Supplemental fat increased intake of C16:0 and C18:0. In preruminant calves, high fat diets stimulate secretion of triglyceride rich lipoproteins such as chylomicrons and very low density lipoproteins (Piot et al., 2000). Metabolism of FA and their subsequent mitochondrial oxidation depends on coordinated induction of enzymes activities involved in FA metabolism at both extramitochondrial level (FA uptake) and mitochondrial level (flux of FA through beta-oxidation) and some of these steps have been shown to be regulated by dietary FA (Pilot et al., 2000). Fatty acids are biologically active molecules that can regulate gene expression, enzyme activities, binding proteins and other cellular processes. Hepatocytes from 7- to 10-d-old calves exposed to 2 mM palmitic acid and 1mM palmitic acid plus 1 mM stearic acid had greater rates of oxidation and ketogenesis of FA compared with all other treatments which included polyunsaturated FA (Mashek and Grummer, 2003). In neonates, FA oxidation rapidly increases after birth to meet energy demands (Girard et al., 1992). Capacity for FA oxidation and ketone body production increases rapidly during the first 24 h after birth (Oden and Treen, 2003;Odle et al., 1995). Plasma concentrations of β-hydroxybutyrate in neonatal calves are low but increase after intake of milk (Senn et al., 2000). Milk intake and carnitine supply is also important for long-chain FA oxidation in brown adipose tissue to ensure heat production by mitochondria uncoupling proteins for thermogenesis and body temperature regulation in neonates of species that contain appreciable amounts of brown fat such as in ruminants (Girard et al., 1992; Hondares et al., 2010).

During trial planning we discussed potential impact of sudden decrease in dietary energy from supplemental fat at completion of the third wk. Previous work has suggested that consistency of milk feeding programs has an impact on calf health and growth (Quigley et al., 2006). Perhaps a gradual decrease in supplemental fat feeding during the second or third wk of the trial may have resulted in more gradual change-over to consuming more starter grain especially for HF calves. Lower border of the zone of thermoneutrality is called the lower critical temperature below which, the animal must increase its rate of heat production to maintain homeothermy. Plane of nutrition likely has a marked effect on thermoneutral heat production and consequently on lower critical temperature. Research reports investigating importance of modulating the environment and diet to prevent cold stress in nursery pigs has shown a clear impact of nutrient use for growth and immune system function (Salak-Johnson and McGlone, 2007). Supplemental energy for low birth weight infants resulted in greater weight gains and greater increases in lean tissue growth than that of control fed infants (Costa-Orvay et al., 2011). There is a paucity of data examining impact of nutrition on growth and health in nursery calves. Work described by Young, (1981) indicated that cold stress was associated with increased resting metabolic rate associated with increased energy requirement for maintenance and stimulation of appetite resulting in an increased rate of passage of digesta resulting in a decrease in digestive efficiency. As a result, cold stressed calves should consume more starter grain, but have a lower efficiency of growth. Net effect of cold stress should be a decrease in efficiency of growth due to a decrease in efficiency of use of dietary energy. Marked changes in efficiency of growth across seasons is likely a result of hormonal and adaptive changes occurring as a result of cold stress.

Dry matter digestibility

Varying the approach to liquid feeding nursery calves, including milk replacer (with or without sodium butyrate vs. whole milk) affected reticulo-rumen and small intestine development, calf growth, and starter intake (Górka et al., 2011). We hypothesized that calves fed supplemental fat would have a less developed rumen at weaning and therefore we anticipated lower apparent total tract starter digestibility in MF and HF compared with LF. Interestingly, apparent starter grain digestibility was 7.0 and 1.6 ± 3.9% greater for MF and HF compared with LF and is possible due to differences in amount of starter intake and thus rate of passage. Development and function of pregastric fermentation was not measured in this study.

Growth and structural development

One objective of feeding supplemental fat is to increase energy intake during the first wk of age when starter intake is expected to be low and not make a substantial contribution towards meeting energy requirements for maintenance and growth. Supplemental fat nearly doubled body weight gain during the first week and differences between control and fat supplemented calves remained through 21 d. Differences in starter intake likely explain a lack of difference in BW gain among treatments prior to weaning and at end of trial. There were essentially no differences in calf structural measurements among treatments except for small but significantly greater hip height and withers height gain through d 56 by LF vs. MF and HF. Balancing dietary energy and protein supply to maintain both body weight gain and structural growth are hallmarks of a successful nursery calf feeding program. These data are in agreement with the review by Khan et al. (2011) which suggest that the optimal milk ration is greater than that used in

conventional practice (restricted feeding) but less than ad libitum energy intake. Younger calves benefit from increased milk rations by being able to express more natural feeding behavior, reduced signs of hunger, improved growth and likely improved health (Khan et al., 2011).

CONCLUSION

Supplemental fat fed during the first 3 wk of the nursery phase in addition to an accelerated feeding program during mild cold stress increased energy intake and gain during the first three weeks but reduced starter intake until weaning. Starter intake by LF calves was greatest until beginning of weaning after which starter intake was similar among treatments. Due to higher starter intake, total intake of metabolizable energy was similar among treatments. Average daily gain during fat supplementation was greater for MF and HF than LF. Lack of increase in BW gain and feed efficiency between MF and HF treatments indicated that HF did not result in advantages over MF. Future research should investigate slower removal of supplemental fat from the milk replacer to prevent large changes in energy intake and evaluate impact of supplemental fat for low birth weight calves.

Table 1. Nutrient composition of milk replacer, fat supplement and starter grain fed to nursery calves during mild cold stress.

	Milk replacer (28:15)	Fat supplement	Starter grain
		% DM	
Crude protein, %	30.9	7.7	21.8
Ether extract, %	15.7	57.4	3.8
ME, Mcal/kg	4.6	6.5	2.8
aNDF, %	<0.01	<0.01	30.1
Ash, %	8.3	4.2	4.23
Calcium, %	1.0	0.3	1.0
Phosphorous, %	0.8	0.3	0.5

Table 2. Fatty acid composition of milk replacer and fat supplement for calves fed 0, 113, or 227 g/d supplemental fat d 2-21 of age.

	Milk replacer	Fat supplement
Fatty acid	g/100 g fatty acids	
C14:0	1.6	3.0
C15:0	0.28	0.20
C16:0	24.6	25.4
C16:1	3.0	3.0
C17:1	0.28	0.30
C18:0	12.9	12.6
C18:1	44.4	42.2
C18:2	10.2	9.8

Table 3. Description of calves fed 0, 113, or 227 g/d supplemental fat d 2-21 of age.

	Treatment[1]				*P*-value		
	LF	MF	HF	SEM	TRT	week	TRT*week
n	27	27	27				
Birth body weight, kg	42.9	41.0	42.1	1.1	0.41	---	---
Ambient temp. at birth, °C	5.5	5.3	5.0	1.1	0.82	---	---
Serum total protein, mg/dL	5.7	5.8	5.8	0.1	0.67	---	---
Parity of dam	1.6	1.8	1.8	0.1	0.28	---	---
Milk replacer offered, kg DM	34.2	32.6	33.5	0.8	0.41	---	---
Total offered (MR + fat), kg DM	34.2[a]	35.1[a]	38.3[b]	0.8	< 0.05	---	---
Nesting score	1.58	1.61	1.64	0.05	0.65	< 0.001	0.95
Fecal score	1.29	1.17	1.24	0.04	0.13	0.13	0.99
Rectal temperature, °C	38.25	38.22	38.25	0.03	0.76	0.09	0.96

[1]Nursery calves were fed one of three treatments; 1) Low fat (LF) (28:15 MR; 2) Medium fat (MF) (28:15 MR + 113 g/d commercial fat supplement (FAT) (60% fat); 3) High fat (HF); 28:15 MR + 227 g/d FAT. MF and HF calves received FAT d 1-21. All calves were fed LF d 22-49. Milk replacer was fed at 1.4% of birth body weight (BBW) d 1-10 and then 1.8% of BBW d 11-42 and 0.9% of BBW d 43-49. Calves were weaned on d 49 and remained in hutches to d 56.

Table 4. Mean intakes of starter, peak starter intake, starter intake expressed as a percentage of body weight, total dry matter intake, feed efficiency and days of age in which calves were consuming selected amounts of starter intake.

	Treatment[1]				*P*-value		
	LF	MF	HF	SEM	TRT	Wk	TRT×Wk
Starter intake, kg/day, d 1-56	1.2[a]	1.0[b]	1.1[ab]	0.06	0.038	<0.001	0.423
Peak starter intake, kg/d	3.5	3.3	3.2	0.1	0.233		
Starter intake, kg/d							
21 d	0.4[a]	0.2[b]	0.1[b]	0.04	0.003		
42 d	1.6[a]	1.3[b]	1.2[b]	0.09	0.045		
49 d	2.3[a]	2.1[ab]	2.0[b]	0.09	0.032		
56 d	3.0	2.9	2.9	0.09	0.646		
Starter intake, % of BW							
21 d	0.7[a]	0.4[b]	0.3[b]	0.07	<0.001		
42 d	2.1[a]	1.7[b]	1.6[b]	0.01	0.009		
49 d	2.9[a]	2.6[b]	2.4[b]	0.1	0.014		
56 d	3.5	3.4	3.3	0.1	0.273		
DMI, kg d 3-49[2]	78.3	70.6	70.6	2.6	0.062		
DMI, kg d 3-56[2]	97.8	90.8	90.5	3.1	0.183		
Gain:feed, kg/d d 3-49	0.52[a]	0.58[b]	0.57[b]	0.02	0.032		
Gain:feed, kg/d d 3-56	0.67	0.69	0.61	0.03	0.175		
Day of age consuming varying amounts of starter							
250 g/d	16.3[a]	21.8[b]	22.7[b]	1.5	0.005		
500 g/d	21.0[a]	26.1[b]	27.9[b]	1.3	0.005		
1000 g/d	31.2[a]	34.4[b]	36.0[b]	1.2	0.012		
2000 g/d	42.9	44.8	45.3	0.8	0.08		
ME_I CMR[4], Mcal/d	2.74	2.69	2.75	0.06	0.775	<0.001	0.999
ME_I fat[5], Mcal/d	0[a]	0.28[b]	0.59[c]	0.04	<0.001	<0.001	<0.001
ME_I CSI[6],Mcal/d	3.16	2.74	2.84	0.2	0.221	<0.001	0.487
Total ME_I[7], Mcal/d	5.87	5.73	6.11	0.2	0.332	<0.001	<0.001

[1]Nursery calves were fed one of three treatments; 1) Low fat (LF) (28:15 MR; 2) Medium fat (MF) (28:15 MR + 113 g/d commercial fat supplement (FAT) (60% fat); 3) High fat (HF); 28:15 MR + 227 g/d FAT. MF and HF calves received FAT d 1-21. All calves were fed LF d 22-49. MR was fed at 1.4% of birth body weight (BBW) d 1-10 and then 1.8% of BBW d 11-42 and 0.9% of BBW d 43-49 Weaned on d 49 and remained in hutches to d 56.

[2]DMI = (starter intake + Milk replacer DM + fat supplement)
[5]Metabolizable energy intake from fat supplement.
[6]Metabolizable energy intake from CSI.
[7]Total metabolizable energy intake per d.

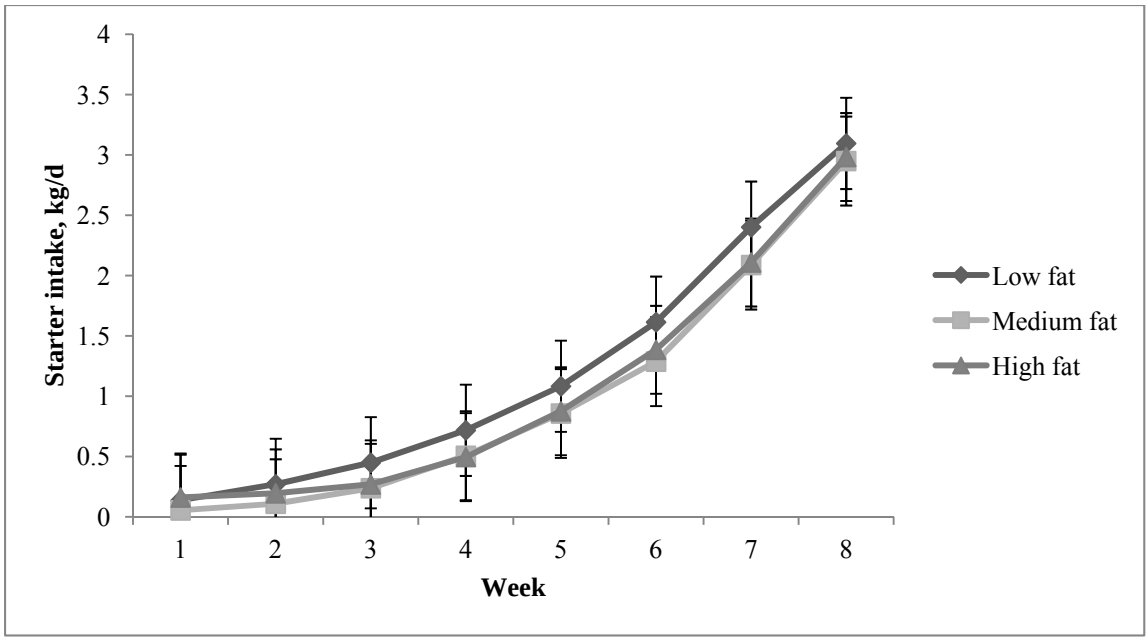

Figure 1. Starter intake during the first 56 d for calves fed milk replacer (MR) with 0, 114 or 227 g/d supplemental fat containing 60% fat, DM basis

[1]Nursery calves were fed one of three treatments; 1) Low fat (LF) (28:15 MR; 2) Medium fat (MF) (28:15 MR + 113 g/d commercial fat supplement (FAT) (60% fat); 3) High fat (HF); 28:15 MR + 227 g/d FAT. MF and HF calves received FAT d 1-21. All calves were fed LF d 22-49. MR was fed at 1.4% of birth body weight (BBW) d 1-10 and then 1.8% of BBW d 11-42 and 0.9% of BBW d 43-49 Weaned on d 49 and remained in hutches to d 56.

Table 5. Least squares means of estimated intakes of crude protein, fat, metabolizable energy and apparent total tract nutrient digestibility from day 2 through day 49 for calves fed increasing amounts of supplemental fat during mild cold stress.

	Treatment[1]				*P* - value
	LF	MF	HF	SEM	TRT
d 3 to 49					
Total CP intake, kg	18.4	17.1	17.2	0.73	0.323
Total fat intake, kg	6.5[a]	7.7[b]	9.1[c]	0.13	< 0.001
d 3 to 56					
Total CP intake, kg	22.6	21.5	21.5	0.91	0.55
Total fat intake, kg	7.2	8.5	9.9	0.16	< 0.001
C16:0 intake from CMR, kg	1.23	1.21	1.24	0.03	0.856
C18:1 intake from CMR, kg	2.2	2.2	2.2	0.06	0.852
C16:0 intake, kg (CMR + suppl. fat)	1.23[a]	1.57[b]	1.96[c]	0.03	<0.001
C18:0 intake, kg (CMR + suppl. fat)	2.22[a]	2.80[b]	3.44[c]	0.06	<0.001
Total tract apparent DMd, %, day 50-52	73.5	80.5	75.1	3.9	0.242

[1]Nursery calves were fed one of three treatments; 1) Low fat (LF) (28:15 milk replacer (MR); 2) Medium fat (MF) (28:15 MR + 113 g/d commercial fat supplement (FAT) (60% fat); 3) High fat (HF); 28:15 MR + 227 g/d FAT. MF and HF calves received FAT d 1-21. All calves were fed LF d 22-49. MR was fed at 1.4% of birth body weight (BBW) d 1-10 and then 1.8% of BBW d 11-42 and 0.9% of BBW d 43-49 Weaned on d 49 and remained in hutches to d 56.

Table 6. Least square means for BW and body measurements during the supplemental fat feeding period (1 to 21 d), at the start of weaning (42 d) and at the end of the trial (56 d) for calves fed increasing amounts of supplemental fat during mild cold stress.

	Treatment[1]				*P*-value
	LF	MF	HF	SEM	TRT
d 2-7 BW gain, kg	2.6[a]	4.4[b]	4.6[b]	0.6	0.026
d 8-14 BW gain, kg	7.5[a]	9.5[b]	9.9[b]	0.7	0.025
d 21					
BW gain, kg	12.4[a]	14.5[b]	15.1[b]	0.7	0.009
Hip height gain, cm	3.2	3.0	2.6	0.4	0.525
Withers height gain, cm	3.7	2.9	3.4	0.5	0.426
Body length gain, cm	1.7	1.3	1.4	0.3	0.698
Heart girth gain, cm	2.7	2.8	2.3	0.3	0.581
Hip width gain, cm	1.5	1.3	1.2	0.3	0.851
ADG, kg/d	0.50[a]	0.82[b]	0.73[b]	0.1	0.022
d 42					
BW gain, kg	33.3	33.1	34.0	1.2	0.855
Hip height gain, cm	8.0	7.4	7.4	0.4	0.551
Withers height gain, cm	7.8	6.7	7.3	0.4	0.215
Body length gain, cm	4.4	3.5	3.7	0.3	0.133
Heart girth gain, cm	6.2	6.5	5.7	0.4	0.289
Hip width gain, cm	3.7	3.6	3.8	0.4	0.979
ADG, kg/d	0.73	0.82	0.82	0.1	0.248
d 56					
BW gain, kg	45.7	46.0	46.1	1.5	0.979
Hip height gain, cm	12.3[a]	10.7[b]	10.3[b]	0.5	0.029
Withers height gain, cm	11.2	9.4	9.9	0.5	0.053
Body length gain, cm	6.2	5.4	5.3	0.4	0.177
Heart girth, gain, cm	8.9	8.5	8.3	0.4	0.510
Hip width, gain, cm	4.5	3.9	3.9	0.4	0.595
ADG, kg/d	0.85	0.82	0.85	0.1	0.821
d 56 total					
BW, kg	89.0	86.0	87.6	2.2	0.60
Hip height, cm	86.5	86.0	86.2	0.6	0.817
Withers height, cm	82.8	83.0	83.0	0.4	0.926
Body length, cm	29.0	29.3	29.3	0.2	0.56
Heart girth, cm	38.2	38.1	38.0	0.3	0.87
Hip width, cm	19.7	19.8	19.8	0.2	0.95

[1] Nursery calves were fed one of three treatments; 1) Low fat (LF) (28:15 MR; 2) Medium fat (MF) (28:15 MR + 113 g/d commercial fat supplement (FAT) (60% fat); 3) High fat (HF); 28:15 MR + 227 g/d FAT. MF and HF calves received FAT d 1-21.

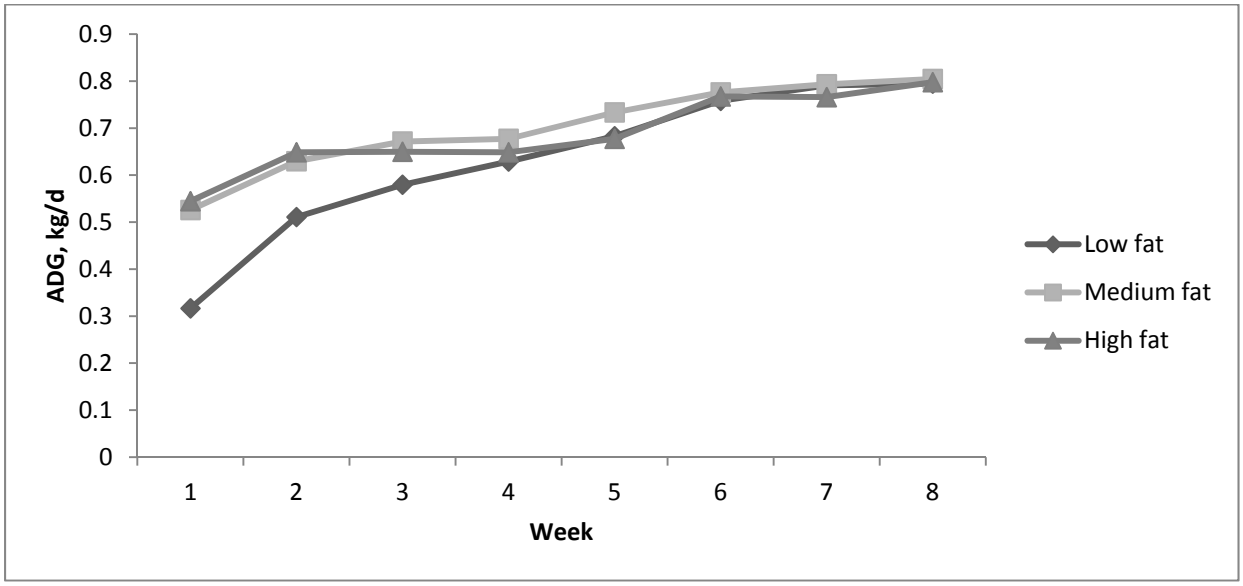

Figure 2. Least squares means for weekly ADG of calves fed one of three treatments; 1) Low fat (LF) (28:15 milk replacer (MR); 2) Medium fat (MF) (28:15 MR + 113 g/d commercial fat supplement (FAT) (60% fat); 3) High fat (HF); 28:15 MR + 227 g/d FAT. MF and HF calves received FAT d 1-21. All calves were fed LF d 22-49. MR was fed at 1.4% of birth body weight (BBW) d 1-10 and then 1.8% of BBW d 11-42 and 0.9% of BBW d 43-49 Weaned on d 49 and remained in hutches to d 56. The largest SEM = 0.08. Effects in model: Treatment $P = 0.430$; week, $P < 0.0001$; treatment × week, $P = 0.077$.

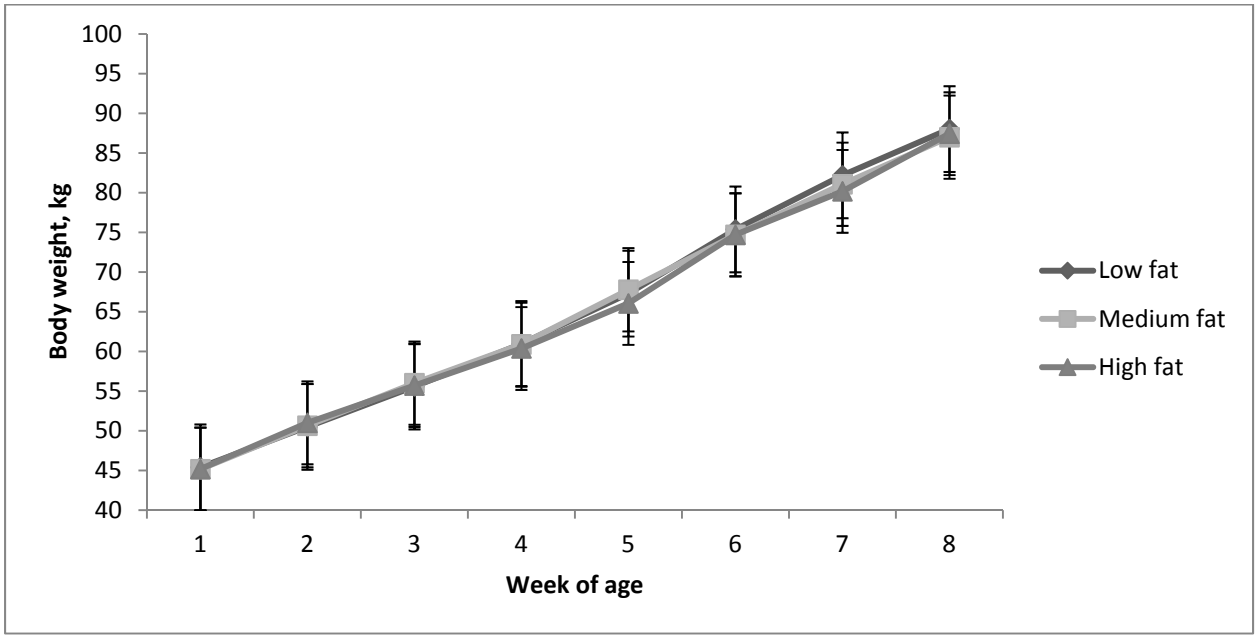

Figure 3. Least squares means of weekly calf body weight. Treatment $P = 0.938$, Week $P < 0.001$, treatment × week $P = 0.533$

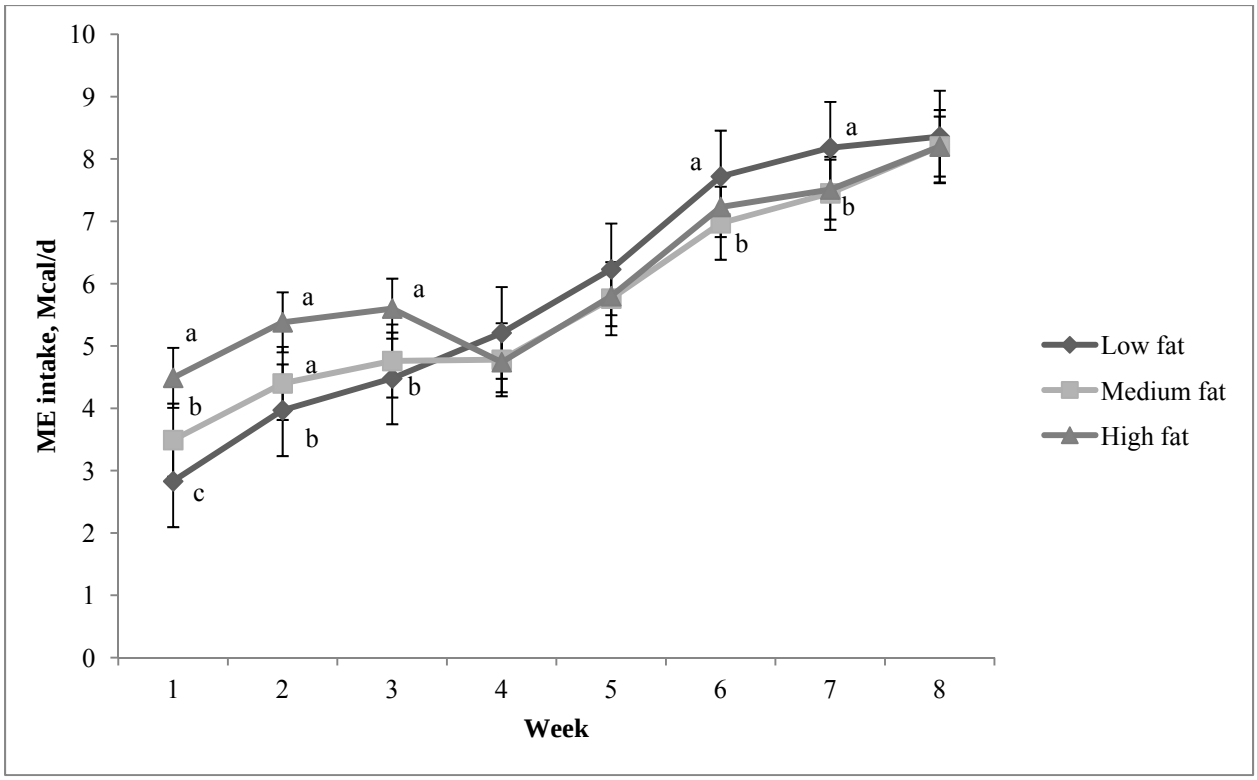

Figure 4. Metabolizable energy intake during the first 56 d for calves fed milk replacer with 0, 114 or 227 g/d supplemental fat containing 60% fat, DM basis. Treatment, $P = 0.330$; week $P < 0.001$; treatment × week $P < 0.001$.

[a,b,c]Means with different superscripts indicate a $P < 0.05$.

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Effect of stocking density in the prepartum period on health and productive parameters

Objectives were to evaluate the effect of different stocking densities during the prepartum period on incidence of diseases and milk yield. Four pens were used in this experiment. Within each replicate (n=3), 2 pens were assigned to 80% stocking density (80D; 38 animals/48 headlocks) and 2 pens were assigned to 100% stocking density (100D; 48 animals/48 headlocks). Nulliparous and parous animals were housed separately pre and postpartum. Animals were scored for body condition and locomotion at enrollment, within 1 d postpartum (DIM), and at 35 and 56 DIM. Cows were examined within 1 DIM for retained placenta; 4, 7, 10, and 14 DIM for metritis; and, 35 DIM for endometritis. Data regarding displacement of abomasum, mastitis, and culling were recorded up to 60 DIM. Cows were milked thrice daily. Data regarding energy corrected milk yield in the first month postpartum is reported. Pen was considered the experimental unit (n=6/treatment). Dichotomous data were analyzed by logistic regression using the GLIMMIX procedure and continuous data were analyzed by ANOVA using the MIXED procedure for repeated measures. Pen was included as the random effect. Treatment was nested within pen and replicate and cows were nested within treatment. Stocking densities were 74.0 and 94.3% (±0.3) of headlocks and 80.7 and 102.8% (±0.4) of stalls for 80D and 100D, respectively. There was no effect of treatment on incidence of stillbirth (80D=3.9 vs 100D=3.4%; P=0.50), retained placenta (80D=4.4 vs 100D=7.4%; P=0.13), and endometritis (80D=7.4 vs 100D=7.1%; P=0.65). There was a tendency (P=0.10) for incidence of metritis to be greater for 80D (21.5%) than 100D (13.9%). Treatment did not affect percentages of cows with locomotion score > 2 at 35 (P=0.94) and 56 (P=0.77) DIM. Body condition score was not affected by treatment (80D=2.97±0.02 vs 100D=2.97±0.01; P=0.91). Percentage of cows removed from the herd within 60 DIM (80D=4.4 vs 100D=3.0%; P=0.42) and yield of energy corrected milk (80D=27.56±1.52 vs 100D=27.98±1.50 kg/d; P=0.85) were not affected by treatment. In conclusion, reducing stocking density did not improve health and productive parameters and surprisingly tended to increase incidence of metritis.

Association of social rank during the prepartum period with health, reproduction, and milk production of dairy cows

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Introduction:

The transition period is a stressful period for the dairy cow. Up to 25 percent of cows leave the herd or die within the first 60 days of milk (Godden et al., 2003). Using social rank may be beneficial in identifying individuals at risk for health and subsequent production problems. Social rank has been associated with the incidence of lameness with low- ranking animals having the greatest risk (Galindo and Broom, 2000). Additionally, low-ranking heifers had a shorter length of estrus and fewer mounts than middle and high-ranking animals which may lead to fewer animals visually observed in estrus (Elkins and Rorie). The objective of this study was to examine social rank during the close up prepartum period and its association with health, reproduction, and milk production during early lactation.

Material and Methods:

The study included 190 prepartum Jersey cows (average lactation 1.7±0.9) and was conducted in a commercial freestall sand-bedded dairy farm in south-central Minnesota for 10 weeks from June to August 2011. Cows were enrolled in the study 4 weeks prior to expected calving date and were balanced for body condition score and those cows with locomotion score > 2 were not included in the study.

Displacements from the feed bunk were measured during 3 h on the day of move-in (d0) at 13:00±1:00 and following fresh feed delivery (05:00±1:00) on d 1, 2, 3 and 7 of each of the wk. A displacement index (Galindo and Broom, 2000) was calculated as the number of displacements initiated by a cow divided by the number of displacements initiated plus number of displacements received by a cow. An averaged displacement index was calculated for each day the cow was observed. Cows with a displacement index of < 0.4 were categorized as low-ranking, 0.4 to 0.6 middle-ranking, and > 0.6 were considered high-ranking. Health events for the first 100 DIM, milk production and composition for the first 3 DHIA tests, and first breeding pregnancy rate were recorded for each cow.

All analysis was conducted with SAS version 9.2. The Logistic procedure was used to evaluate associations of social ranking to health and reproductive events. The Mixed procedure was used to analyze milk and milk composition. Animal was used as a random effect. Least square means were separated with the PDIFF statement. Other covariates tested in all models included lactation and pen.

Results:

Eighty-nine animals were considered low-ranking with 59 and 42 in the middle and high-ranking groups, respectively. There was no association of social rank with retained placenta, metritis, death, displaced abomasum, and mastitis events. Displacement index was associated with first breeding pregnancy rate (*P*

< 0.01). Middle-rank cows were 3 times more likely to become pregnant after first AI than low-ranking cows with no differences between low ranking and high ranking cows.

There was no association between milk production and social rank. Percent milk fat from the second test was associated with social rank (P = 0.04). Milk fat percentage was greater in low-ranking cows than high-ranking cows (4.1±0.13 vs. 3.7±0.16%, respectively). Middle-ranking cows, however, had similar milk fat percentage to low and high-ranking cows.

In conclusion, social rank in the prepartum period was only associated with pregnancy status and 2nd test milk fat percentage in early lactation dairy cows. There were no associations of social rank and examined health parameters.

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